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# Fishery Bulletin

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# Fishery Bulletin

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# Analysis of sex-specific spawning biomass per recruit of the sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan

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**Abstract**—Analyses of sex-specific yield per recruit and spawning stock biomass per recruit were conducted to evaluate the current status of the sailfish (*Istiophorus platypterus*) fishery in the waters off eastern Taiwan. Natural mortality rates estimated from Pauly's empirical equation were 0.26/yr for females and 0.27/yr for males. The current fishing mortality rates were estimated as 0.24/yr and 0.43/yr for females and males, respectively, which are much lower than the estimated $F_{0.1}$ (0.62/yr and 0.79/yr for females and males, respectively) and $F_{SSB40}$ (0.46/yr for females) which are commonly used as target reference points in fisheries management. The effects of the fishing mortality, natural mortality, and age at first capture on the estimates of biological reference points were evaluated by using the Monte Carlo simulation. The results indicate that failure to consider the uncertainty in parameters such as natural mortality or age at first capture may lead to the improper estimation of biological reference points. This study indicates the possibility of current fishing mortality exceeding the target biological reference points may be negligible for sailfish in the waters off eastern Taiwan. However, in view of the recent rapid increase in fishing effort, it is evident that the stock status and development of the fishery need to be closely monitored.

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Sailfish (*Istiophorus platypterus*) is a circumtropically distributed species (Hoolihan, 2005). Sailfish is a member of the billfish family, Istiophoridae, which also includes marlins and spearfishes, and is considered a bycatch species in commercial fisheries. Off the eastern coast of Taiwan, sailfish are economically important and seasonally abundant from April to October (abundance peaks from May to July). Sailfish are mainly caught by drift gill nets, although some are also caught by set nets, harpoons, and as incidental bycatch in inshore longline fisheries (Chiang, 2004). There are virtually no discards of sailfish in Taiwan. For the past decade, the annual landings of sailfish off Taiwan waters have fluctuated between 500 and 1000 metric tons, of which over 50% have come from waters off Taitung (eastern Taiwan).

Globally, large predatory fish species, including billfish, are declining at alarming rates because of excessive exploitation (Myers and Worm, 2003). Recent increases in the exploitation of billfish stocks by both commercial and recreational fisheries clearly point to the need for accurate assessments, if the goal is to develop sustainable billfish fisheries (Uozumi, 2003). However, because few fisheries target sailfish, assessments have not been conducted, resulting in few or no effective management measures. For sailfish in the Pacific Ocean and Indian Ocean, no assessments have been conducted, and stock status remains unknown. Historical sailfish catch data from the Pacific Ocean are scant, as are data on the length and age composition of the catch. This lack of data precludes the use of most stock assessment tools such as production models and age-structured models (Punt, 1997; Prager and Goodyear, 2001; Liu et al., 2006). However, recent biological studies on sailfish in the waters off eastern Taiwan (Chiang et al., 2004, 2006) have provided an opportunity to apply yield per recruit ($Y/R$) and spawning biomass per recruit ($SSB/R$) models (Govender, 1995; Griffiths, 1997) to estimate biological reference points including the fishing mortality rate corresponding to the point where the slope of the yield-per-recruit curve

equals 10% of the slope at the origin ($F_{0.1}$; Gulland and Boerema, 1973) and the fishing mortality rate corresponding to a specific percentage ($x$%) of the spawning biomass per recruit at the unfished level ($F_{SSBx}$) for the eastern Taiwan sailfish stock. The status of the fishery for this stock could be examined by comparing the fishing mortality at the current level ($F_{CUR}$) with the biological reference points.

A per-recruit analysis requires information on growth, mortalities, and selectivity of fishing gear. Catch curve analysis (Ricker, 1975) is the most common method employed for estimating total mortality when data on the age composition of catch are available. For a specified natural mortality, $F_{CUR}$ could be computed simply by subtracting the natural mortality from the total mortality. However, in most cases large uncertainty is associated with the estimation of natural mortality and other life history parameters, which can lead to large uncertainty in the estimation of $F_{CUR}$ and biological reference points.

The objective of this study was to evaluate the current status of the sailfish fishery in waters off eastern Taiwan by comparing the current fishing mortality rate (estimated from analyzing length composition data collected from the fishery) with the biological reference points derived from the per-recruit analyses (Butterworth et al., 1989; Sun et al., 2002, 2005). In addition, a Monte Carlo simulation study was conducted for evaluating the influence of uncertainty associated with mortalities and the age at first catch ($t_c$) on the estimation of biological reference points. This study provides an approach that can be used to assess the status of fisheries for which limited information does not allow us to conduct a full stock assessment.

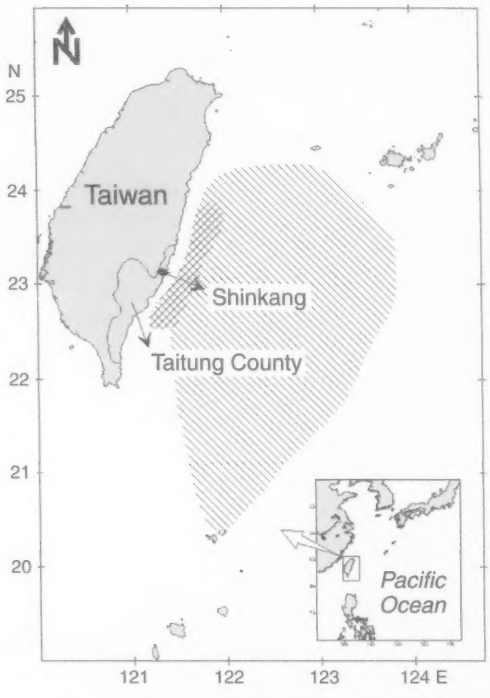


**Figure 1**

The fishing grounds where sailfish (*Istiophorus platypterus*) are caught as bycatch in the gillnet, harpoon, and longline fisheries based at the Shinkang fishing port of Taiwan. Crosshatched area is where the gillnet and harpoon fisheries take place and the longline fishery takes place in larger area indicated by oblique lines. Samples were collected during the period from July 1998 to July 2005 to estimate biological metrics for per-recruit analyses.

## Materials and methods

### Length and age composition of the catch

Length composition data were obtained by measuring sailfish landed at the Shinkang fish market in eastern Taiwan (Fig. 1) during the period from July 1998 to July 2005. Specimens were randomly selected from the landings and measured for their lengths and weights. The sex of each specimen was identified from the appearance of its gonads. Samples of the first dorsal fin were taken from 1166 of the sampled individuals for which lengths were measured and used to age the sailfish (Chiang et al. 2004). These subsampled fish were used to construct sex-specific age-length keys, which in turn were used to convert the length-frequency data into age-composition data.

### Estimating mortality rates

For each sex, the dynamics of a simulated year class can be projected forward from one year to another by using the exponential survival equation (Ricker, 1975):

$$N_{t+1} = N_t e^{-(M+FS_t)}, \quad (1)$$

where $N_t$ = the number of fish at the beginning of age $t$;

$M$ = the instantaneous natural mortality rate;

$F$ = $t$ the fishing mortality of fully-recruited fish; and

$S_t$ = the fishing gear selectivity of fish at age $t$.

Selectivity is the relative vulnerability of different age or size classes to the fishing gear. In this study, we assumed that the selectivity follows a dome-shaped distribution because our length-frequency data were mostly collected from gill nets. This dome-shaped selectivity can be quantified with the following normal distribution density function:

$$S_t = \frac{1}{\sqrt{2\pi}\sigma} e^{\frac{-(t-\mu)^2}{2\sigma^2}}, \quad (2)$$

where $\mu$ = the age at the mode of the dome-shaped selectivity; and
$\sigma$ = the standard deviation of the dome-shaped selectivity.

The expected catch ($\hat{C}_t$) of fish at age $t$ can be estimated based on the catch equation (Ricker, 1975):

$$\hat{C}_t = \frac{FS_t}{M+FS_t} N_t (1-e^{-(M+FS_t)}), \quad (3)$$

The parameters of $N_0$, $F$, $\mu$, and $\sigma$ can be estimated simultaneously by minimizing the following composite objective function:

$$\sum_t (C_t - \hat{C}_t)^2 + (\max(S_t)-1)^2, \quad (4)$$

where $C_t$ = the observed numbers of catch at age $t$.

The $F$ estimated above was considered as the current fishing mortality ($F_{CUR}$) in this study. The approach outlined above for the catch-curve analysis is similar to the one described in Rudershausen et al. (2008).

A total of 1000 independent bootstrap samples of $F$ were derived from 1000 sets of length-frequency data drawn randomly with replacement from the individuals of original length-frequency data.

Pauly's (1980) empirical equation was used to estimate $M$ for each sex, and the mean sea surface temperature around eastern Taiwan waters fitted to the equation was about 26°C.

### Per-recruit analyses

Yield per recruit ($Y/R$) of sailfish in the waters off eastern Taiwan was estimated from the following model:

$$Y/R = \sum_{t=t_c}^{t_{max}} \left( \overline{W}_t \times \frac{F_t}{Z_t} \times \left(1-e^{-(FS_t+M)}\right) \times e^{-\sum_{i=0}^{t-1}(FS_i+M)} \right), \quad (5)$$

where $\overline{W}_t$ = the mean weight of fish at age $t$; and
$t_c$ = the age at first capture.

Mean weight at age was computed as a power function of midyear lower jaw fork length ($L_t$):

$$W_t = a \times L_t^b, \quad (6)$$

and midyear lower jaw fork length was estimated from the von Bertalanffy growth function

$$L_t = L_\infty \left(1-e^{-K(t+0.5-t_0)}\right), \quad (7)$$

where $K$ = the growth parameter;
$L_\infty$ = the average asymptotic length; and
$t_\infty$ = hypothetic age at length of 0 (Ricker, 1975; see Table 1).

**Table 1**

Biological parameters used in the per-recruit analysis for the sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan during the period from July 1998 to July 2005. VBGF is the von Bertalanffy growth function, $L_\infty$ = the asymptotic length, $K$ = the growth coefficient, $t_0$ = the hypothetical age at length zero; Length-weight relationship is $W = A \times L^B$, where $W$ = rought weight (in kg) and $L$ = lower jaw fork length (in cm); Maturity fraction parameters $r_m$ = the slope of logistic equation fitted to the maturity data collected, and $t_m$ = the age at 50% sexual maturity.

| Parameter | Female | Male |
|---|---|---|
| VBGF | | |
| $L_\infty$ | 250.29 cm | 240.539 cm |
| $K$ | 0.138/yr | 0.145/yr |
| $t_0$ | –2.99 | –2.781 |
| Length-weight relationship | | |
| $A$ | $2.3234\times10^{-6}$ | $1.1933\times10^{-5}$ |
| $B$ | 3.1013 | 2.7828 |
| Maturity fraction | | |
| $r_m$ | 1.525 | |
| $t_m$ | 5 | |

The maximum lifespan ($t_{max}$) of sailfish in the waters off eastern Taiwan was unknown but was estimated by using the empirical relationship of Taylor (1958):

$$t_{max} = t_0 + \frac{2.996}{K}, \quad (8)$$

The equation for spawning stock biomass per recruit ($SSB/R$) is

$$SSB/R = \sum_{t=t_m}^{t_{max}} \left( fr_t \times W_t \times e^{-\sum_{i=t_r}^{t-1}(F_i+M)} \right), \quad (9)$$

where $W_t$ = the mean weight at age $t$ that was calculated from the von Bertalanffy function and length-weight relationship for female sailfish; and
$fr$ = the fraction of female sailfish that are mature.

In this case $fr$ is represented by a logistic equation fitted to maturity data collected from sailfish caught in the eastern waters off Taiwan (Chiang et al., 2004, 2006). The logistic equation can be written as

$$fr_t = \frac{1}{1+e^{-r_m(t-t_m)}}, \quad (10)$$

where $r_m$ = the slope of the logistic curve; and
$t_m$ = age at which 50% of fish are mature.

**Table 2**

Scenarios designed to examine the effects of uncertainty of $F$, $M$, and $t_c$ on the estimates of biological reference points of sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan. Scenarios A–D were used to evaluate the effects of changes in a single parameter, scenarios E–H were used to evaluate the results of changes in combinations of two parameters, and scenarios I and J were used to evaluate the results when three parameters were subject to uncertainty. ($\sigma_F$=standard deviation from the bootstrapped estimation; $M$ = natural mortality per year; $t_c$ = age at first catch)

| | Parameters | | | | |
|---|---|---|---|---|---|
| | $\sigma_F$ | | $M$ | | |
| Scenario | Female | Male | Female | Male | $t_c$ |
| Base | 0 | 0 | 0.26 | 0.27 | 5 |
| A | 0.046 | 0.045 | 0.26 | 0.27 | 5 |
| B | 0 | 0 | 0.2–0.3 | 0.2–0.3 | 5 |
| C | 0 | 0 | 0.15–0.35 | 0.15–0.35 | 5 |
| D | 0 | 0 | 0.26 | 0.27 | 5–7 |
| E | 0.046 | 0.045 | 0.2–0.3 | 0.2–0.3 | 5 |
| F | 0.046 | 0.045 | 0.15–0.35 | 0.15–0.35 | 5 |
| G | 0 | 0 | 0.2–0.3 | 0.2–0.3 | 5–7 |
| H | 0 | 0 | 0.15–0.35 | 0.15–0.35 | 5–7 |
| I | 0.046 | 0.045 | 0.2–0.3 | 0.2–0.3 | 5–7 |
| J | 0.046 | 0.045 | 0.15–0.35 | 0.15–0.35 | 5–7 |

### Biological reference points

The following biological reference points were estimated in order to determine the current status of the sailfish fishery: $F_{0.1}$, $F_{SSB25}$, and $F_{SSB40}$. $F_{SSB25}$, and $F_{SSB40}$ are fishing mortality rates corresponding to the 25% and 40% of the spawning biomass per recruit at unfished level. The choice of 25% or 40% was relatively arbitrary for the fishery, but these values have been used as different levels of reference points for other relatively long-lived marine fishes (e.g., Griffiths, 1997; Kirchner, 2001; Sun et al., 2002, 2005). The spawning potential ratio ($SPR$) is the $SSB/R$ at a given fishing mortality divided by the $SSB/R$ without fishing (Gabriel et al., 1989; Goodyear, 1993; Katsukawa et al., 1999; Watanabe et al., 2000; Sun et al., 2002, 2005) and can be calculated as

$$SPR = \frac{SSB/R}{SSB/R\big|_{F=0}}. \quad (11)$$

Several authors have advocated designating $F_{0.1}$ or $F_{SSB40}$ as target reference points and $F_{SSB25}$ as a threshold reference point in order to obtain near optimal yields while minimizing the likelihood of stock collapse (Gulland and Boerema, 1973; Deriso, 1987; Hildén, 1993; Sun et al., 2002, 2005). We adopted these target and threshold reference points in this study.

### Simulation study

The Monte Carlo simulation approach was applied to evaluate the sensitivity of estimating biological reference points with respect to parameters $F$, $M$, and $t_c$. To quantify the uncertainty of $F$, $F$ was assumed to follow a normal distribution with a mean and standard deviation ($\sigma_F$)—the latter estimated from the bootstrapped estimation of $F$. However, there was no information on the distribution of $M$ and $t_c$. We assumed a uniform distribution for $M$ and $t_c$ by referring to the estimation of Pauly's empirical equation and the age at full recruitment from the age composition of sailfish in the waters off eastern Taiwan. The values of $M$ and $t_c$ were sampled randomly from the corresponding uniform distributions defined in Table 2. Ten scenarios were designed to examine the effects of different combinations of the uncertain in parameters $F$, $M$, and $t_c$ on the estimation of biological reference points (Table 2). Scenarios A–D were used to evaluate the effects of changes in a single parameter, scenarios E–H were used to evaluate the results of changes in combinations of two parameters, and scenarios I and J were used to evaluate the results when three parameters were subject to uncertainty. For each scenario, 100 replicates of biological reference points were estimated by using the parameters of $F$, $M$, or $t_c$ randomly drawn from their assumed distributions. The median and the interquartile range were used to quantify the central tendency and variation for the distributions of estimated biological reference points.

## Results

### Age composition

Length data were obtained for 12,323 sailfish (3532 females and 8791 males), and age data for 1166 of these

fish (446 females and 720 males). The range of lower jaw fork length was 80–239 cm for females and 78–227 cm for males (Fig. 2). Age compositions of samples collected during the entire study period indicated that most sailfish caught off eastern Taiwan are larger than the age at 50% maturity, and peak in length at 5 years (Fig. 3). Accordingly, the estimates of age at full recruitment ($t_c$) and the sample size during studying periods are shown in Table 3. The estimates of $t_c$ varied from 5 to 7 years for different time periods. Few sampled fishes were older than 11 years for both sexes and hence age 12 and higher were combined into the 12+ group (Fig. 3). The empirical estimates of maximum lifespan ($t_{max}$) were 13 years for males and 21 years for females.

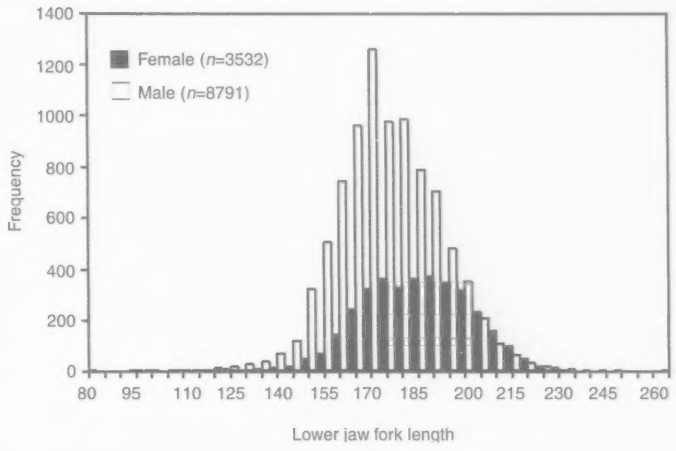


**Figure 2**

The length-frequency distributions (5-cm intervals) of female (black bars) and male (white bars) sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan during the period from July 1998 to July 2005. Most fish caught were 160–190 cm in lower jaw fork length.

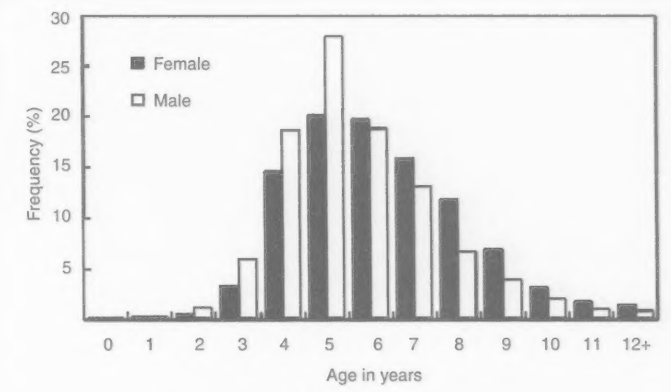


**Figure 3**

The age-frequency distributions for female (black bars) and male (white bars) sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan during the period from July 1998 to July 2005. Most fish caught were 4–7 years old.

### Mortality rates

The estimates of $F$ were 0.24/yr for females and 0.43/yr for males based on the samples collected during the entire study period. Based on bootstrap analysis, the standard deviation of $F$ was 0.046/yr and 0.045/yr for females and males, respectively. In addition, 79% of bootstrap replicates of $t_c$ were 5 years old and few were 6 or 7 years old. The values of $M$ estimated from the Pauly's empirical equation were 0.26/yr for females and 0.27/yr for males. In this study, therefore, $F$ of 0.24/yr for females and 0.43/yr for males, $t_c$ of 5 years and $M$ of 0.26/yr for females and 0.27/yr for males were set as the base case values for the subsequent analyses. Sensitivity analyses were also conducted for examining the results of $Y/R$ and $SPR$ analyses by assuming values of 0.20, 0.30, and 0.35 for $M$.

### *Y/R* and *SPR* models and biological reference points

In this study, selectivities for females and males were assumed to be dome-shaped, and the estimated selectivity curves are shown in Figure 4. The estimates of $F_{CUR}$, $F_{0.1}$, and $Y/R$ under various values of $M$ are summarized in Table 4. For the base case, $F_{CUR}$ (0.24/yr for females and 0.43/yr for males) were substantially lower than the corresponding biological reference points $F_{0.1}$ (0.62 /yr for females and 0.79/yr for males). The estimates of $Y/R_{CUR}$ were 3.37 kg for females and 3.72 kg for males and the estimates of $Y/R_{0.1}$ were 5.11 kg for females and 4.68 kg for males (Fig. 5). Even in the most conservative case, when $M$ was assumed to be 0.2, $F_{CUR}$ was still lower than $F_{0.1}$ for both females and males.

The effect of varying $t_c$ on $Y/R$ is shown in Figures 6 and 7. At low levels of $F$, $Y/R$ generally increased rapidly over the range of $t_c$ values tested. The values of $t_c$ that maximized the yield per recruit decreased with the magnitude of $M$ and increased with the level of $F$ but typically ranged between 2 and 5 years for females and males.

**Table 3**

Sample sizes and estimates of age at full recruitment for female and male sailfish (*Istiophorus platypterus*) by year in the waters off eastern Taiwan during the period from July 1998 to July 2005.

| | Sample size | Age at full recruitment | | Sample size | Age at full recruitment |
|---|---|---|---|---|---|
| Female | | | Male | | |
| 1998 | 131 | 6 | 1998 | 525 | 6 |
| 1999 | 702 | 5 | 1999 | 1943 | 5 |
| 2000 | 387 | 7 | 2000 | 578 | 7 |
| 2001 | 358 | 6 | 2001 | 730 | 5 |
| 2002 | 349 | 7 | 2002 | 576 | 5 |
| 2004 | 484 | 5 | 2004 | 1123 | 5 |
| 2005 | 1121 | 6 | 2005 | 3316 | 5 |
| Overall | 3532 | 5 | Overall | 8791 | 5 |

**Table 4**

Estimates of current fishing mortality ($F_{CUR}$), current yield per recruit ($Y/R_{CUR}$), and the reference points of $F_{0.1}$ and $Y/R_{0.1}$ at different levels of natural mortality ($M$) for female and male sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan during the period from July 1998 to July 2005. $F_{0.1}$ = the fishing mortality rate corresponding to $Y/R_{0.1}$; $Y/R_{0.1}$ = the point of a yield-per-recruit curve where the slope equals 10% of the slope at the origin.

| $M$ (1/yr) | $F_{CUR}$ (1/yr) | $F_{0.1}$ (1/yr) | $Y/R_{CUR}$ (kg) | $Y/R_{0.1}$ (kg) |
|---|---|---|---|---|
| Female | | | | |
| 0.20 | 0.26 | 0.54 | 5.36 | 7.08 |
| 0.26 | 0.24 | 0.62 | 3.37 | 5.11 |
| 0.30 | 0.22 | 0.69 | 2.45 | 4.13 |
| 0.35 | 0.20 | 0.80 | 1.63 | 3.19 |
| Male | | | | |
| 0.20 | 0.46 | 0.70 | 5.79 | 6.66 |
| 0.27 | 0.43 | 0.79 | 3.72 | 4.68 |
| 0.30 | 0.41 | 0.83 | 3.06 | 4.04 |
| 0.35 | 0.38 | 0.91 | 2.21 | 3.17 |

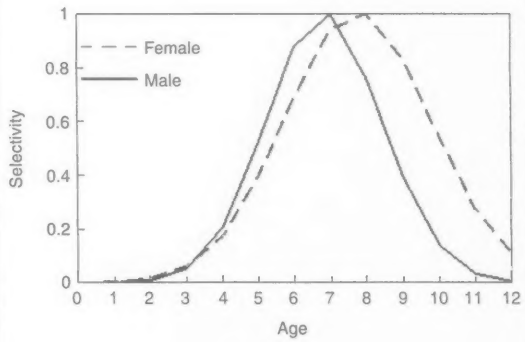


**Figure 4**

The estimated selectivity curves for female (dashed line) and male (solid line) sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan. Selectivity is the relative vulnerability of different age or size classes to the fishing gear. In this study, selectivity is assumed to be dome shaped because length-frequency data were mostly collected from gillnets.

The rapid growth and relatively late $t_c$ (5 years) of sailfish in the waters off eastern Taiwan produced low $Y/R$ and high $SPR$ at current fishing mortalities compared with reference points (Figs. 4 and 7). The estimates of $F_{SSB25}$, $F_{SSB40}$, $SPR$ and $Y/R$ under the various values of $M$ are summarized in Table 5 and Figure 8. The base-case estimate of $F_{CUR}$ for females (0.24/yr) was lower than the corresponding reference points $F_{SSB40}$ (0.46/yr) and $F_{SSB25}$ (0.94/yr); the current SPR was estimated to be about 57.20% of its unfished level. Under the low value of $M$ (0.2/yr), the estimate of $F_{CUR}$ for females (0.26/yr) was lower than $F_{SSB40}$ (0.36/yr) and substantially lower than $F_{SSB25}$ (0.67/yr). Increasing $t_c$ to older than six years of age would ensure that the SPR was maintained at a value higher than the threshold level (i.e., 25% of its unfished level) at almost any level of fishing mortality (Fig. 9) with relatively little effect on $Y/R$.

### Simulation scenarios

The box plots of the estimates of $F_{0.1}$ and $F_{CUR}/F_{0.1}$ are shown in Figure 10 for scenarios A–J with the assumption of uncertainty in $F$, $M$, and $t_c$ defined in Table 2. Although the medians of these two quantities were close to those of the base case for most scenarios, the variations of these quantities were diverse depending on the assumptions of uncertainty of the parameters. For scenarios A–D with only one parameter subject to uncertainty, the estimates of $F_{0.1}$ were independent of

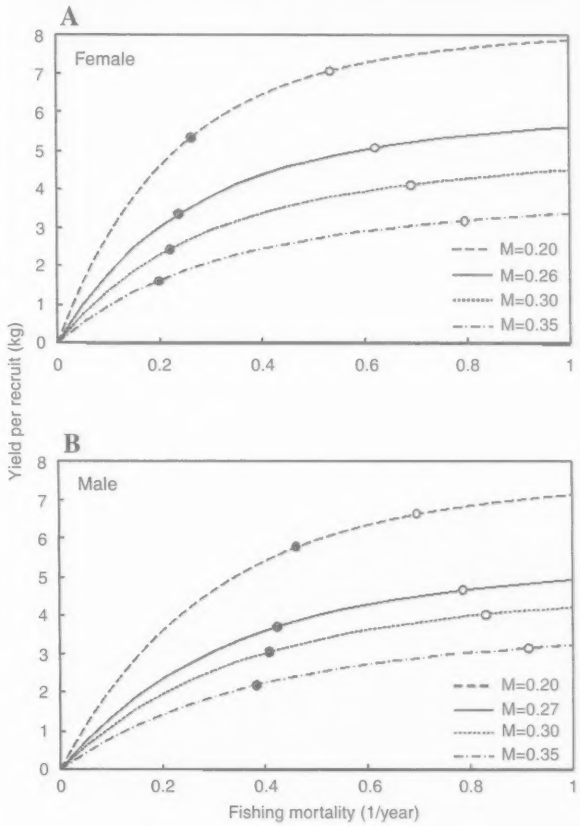


**Figure 5**

Yield per recruit ($Y/R$) against fishing mortality per year for female (**A**) and male (**B**) sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan. ● = $F_{CUR}$, and ○ = $F_{0.1}$.

**Table 5**

Estimates of current fishing mortality ($F_{CUR}$) and the reference points of $SSB/R_{SSBx}$ and $SPR$ at different levels of natural mortality ($M$) for female sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan during the period from July 1998 to July 2005. $F_{SSBx}$ = the fishing mortality rate corresponding to $SSB/R_{SSBx}$; $SSB/R_{SSBx}$ = a specific percentage ($x$%) of the spawning biomass per recruit at the unfished level; SPR (spawning potential ratio) = the $SSB/R$ at a given fishing mortality divided by the $SSB/R$ without fishing.

| M (1/yr) | $F_{CUR}$ (1/yr) | $F_{SSB40}$ (1/yr) | $F_{SSB25}$ (1/yr) | $SSB/R_{CUR}$ (kg) | $SSB/R_{SSB40}$ (kg) | $SSB/R_{SSB25}$ (kg) | $SPR_{CUR}$ (%) |
|---|---|---|---|---|---|---|---|
| 0.20 | 0.26 | 0.36 | 0.67 | 32.50 | 26.56 | 16.60 | 48.96 |
| 0.26 | 0.24 | 0.46 | 0.94 | 22.22 | 15.53 | 9.71 | 57.20 |
| 0.30 | 0.22 | 0.54 | 1.22 | 17.42 | 11.14 | 6.96 | 62.57 |
| 0.35 | 0.20 | 0.70 | 1.73 | 12.97 | 7.52 | 4.70 | 68.95 |

the changes in $F$ but were very sensitive to the uncertainty in $M$. Adding the uncertainty in $t_c$ resulted in slightly higher estimates of $F_{0.1}$ than that for the base case. Higher estimates of $F_{0.1}$ could have resulted from $t_c$ with values larger than 5 years (base case) selected in this scenario. In the case of this study, higher $t_c$ pro-

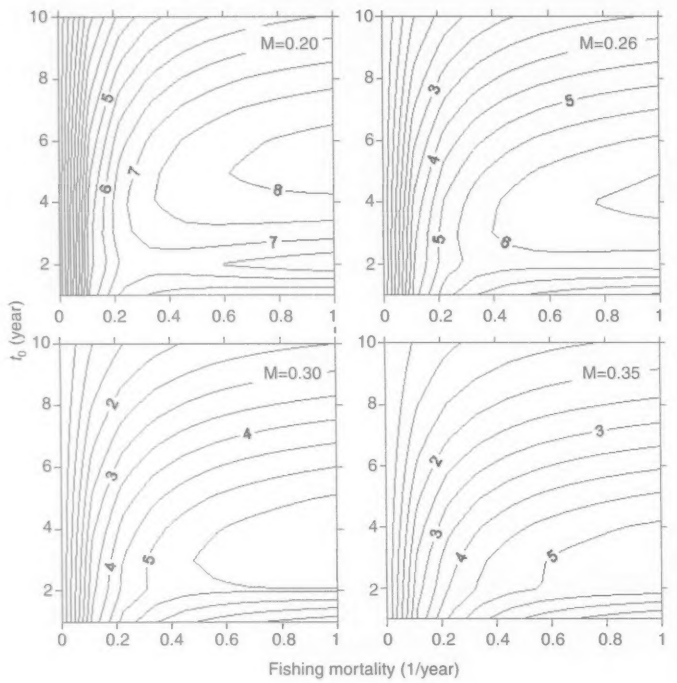


**Figure 6**

Yield-per-recruit isopleths against fishing mortality per year and age at first capture ($t_c$) at different levels of natural mortality ($M$) for female sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan.

duced higher estimates of $F_{0.1}$ for sailfish in the water of eastern Taiwan. For scenarios E–J that incorporated the combinations of uncertainties in $F$, $M$, and $t_c$, large variations were observed for estimates of $F_{0.1}$ when a higher level of uncertainty was assumed for $M$ (scenarios F, H, and J). Even though combinations of parameters with higher uncertainties were considered, the upper bounds (the third quartile) of $F_{CUR}/F_{0.1}$ were lower than 1 for all scenarios. This result implied that it was highly unlike that $F_{CUR}$ could exceed $F_{0.1}$. In contrast, the effects of adding uncertainties in $F$ and $t_c$ were relatively minor.

Figure 11 shows the box plots of the estimates of $F_{SSB40}$, $F_{SSB25}$, $F_{CUR}/F_{0.1SSB40}$, and $F_{CUR}/F_{0.1SSB25}$ for scenarios A–J (with uncertainty). Similarly, higher levels of uncertainties in $M$ resulted in higher variations for the estimates of $F_{SSB40}$ and $F_{SSB25}$ (scenarios C, F, H, and J). Moreover, the estimates of $F_{SSB40}$ and $F_{SSB25}$ were obviously higher than those of the base case when the uncertainty in $t_c$ was considered (scenarios D, G, H, I, and J), which might result from female fish younger than $t_c$ being less vulnerable to fishing gear. Therefore, higher values of $SSB/R$ would be obtained when larger values of $t_c$ were selected and higher estimates of $F_{SSB40}$ and $F_{SSB25}$ were revealed for these scenarios. For all scenarios, the medians of $F_{CUR}/F_{SSB40}$ and $F_{CUR}/F_{SSB25}$ were 0.71 and 0.37, respectively, which were similar to those of the base case (Table 5). The upper boundaries of $F_{CUR}/F_{SSB40}$ and $F_{CUR}/F_{SSB25}$ were substantially lower than 1 for all the scenarios and no simulation runs were observed to have the values higher than 1.

## Discussion

### Mortality rates

The $Y/R$ curve is so flat-topped that maximum $Y/R$ cannot be well defined and may not be attained under any practical fishing mortality rate (Fig. 5). Reference points such as $F_{0.1}$, $F_{SSB25}$, and $F_{SSB40}$ have often been used to develop fishery management strategies. Previous studies have indicated that $Y/R$ and $SPR$ are sensitive to values of $M$ (Griffiths, 1997; Kirchner, 2001; Sun et al., 2005). In this study, however, the estimates of $F_{CUR}$ were much lower than the target levels of $F_{0.1}$ or $F_{SSB40}$, except for the scenario when $M$ was assumed at the lower bound of 0.2. Moreover, the estimates of $F_{CUR}$ were never larger than the threshold level of $F_{SSB25}$ even

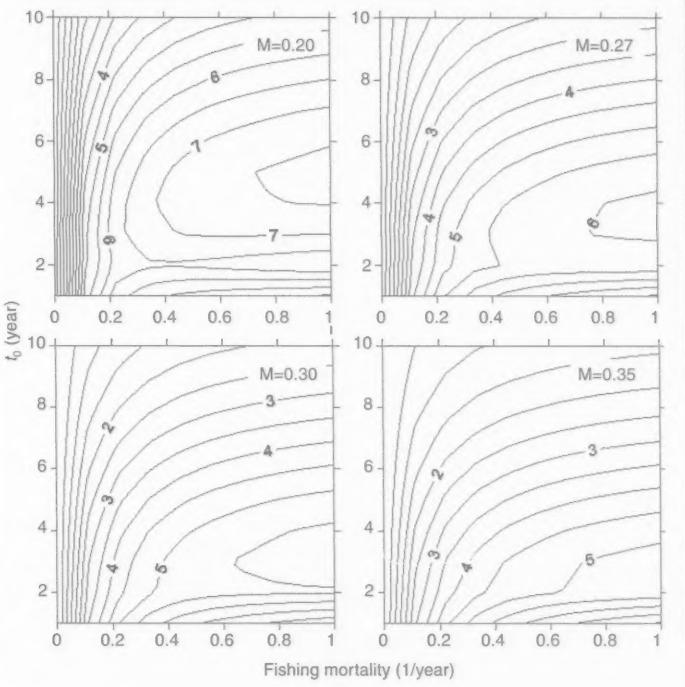


**Figure 7**

Yield-per-recruit isopleths against fishing mortality per year and age at first capture ($t_c$) at different levels of natural mortality ($M$) for male sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan.

when $M$ was assumed to be 0.2. This result would indicate that the stock of sailfish in the waters off eastern Taiwan appears to be moderately exploited and has relative low risk of being overfishing.

### Sex-specific per-recruit analyses and sensitivity analyses

Most assessment methods require historic information on catch, effort, and catch-at-age (or catch-at-length). The lack of long-term fishery statistics usually makes it difficult to evaluate the status of populations exploited by small-scale fisheries or taken incidentally (e.g., Govender, 1995; Barbieri et al., 1997; Griffiths, 1997; Jones and Wells, 2001; Sun et al., 2002, 2005). Per-recruit analyses, which require only parameters related to life span and mortality, combined with an analysis of catch curves, can become an alternative method for evaluating the status of a fishery. Because per-recruit analyses can provide the estimates of biological reference points and the catch curve analysis can yield

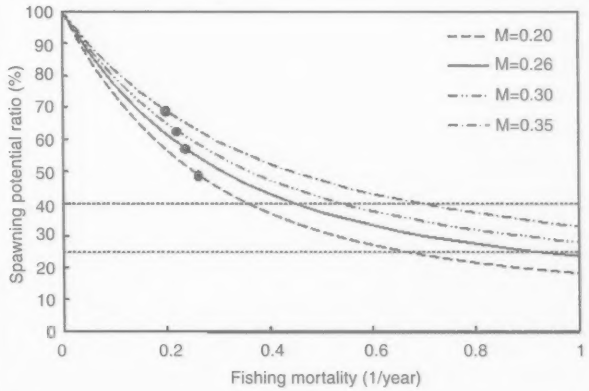


**Figure 8**

Spawning potential ratio (expressed as a percentage) against fishing mortality for female sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan where age at first capture ($t_c$) = 5 years (solid dots are $SPR_{CUR}$ levels; horizontal dashed lines are levels for the biological reference points of $F_{SSB40}$ and $F_{SSB25}$, and ● = $F_{CUR}$).

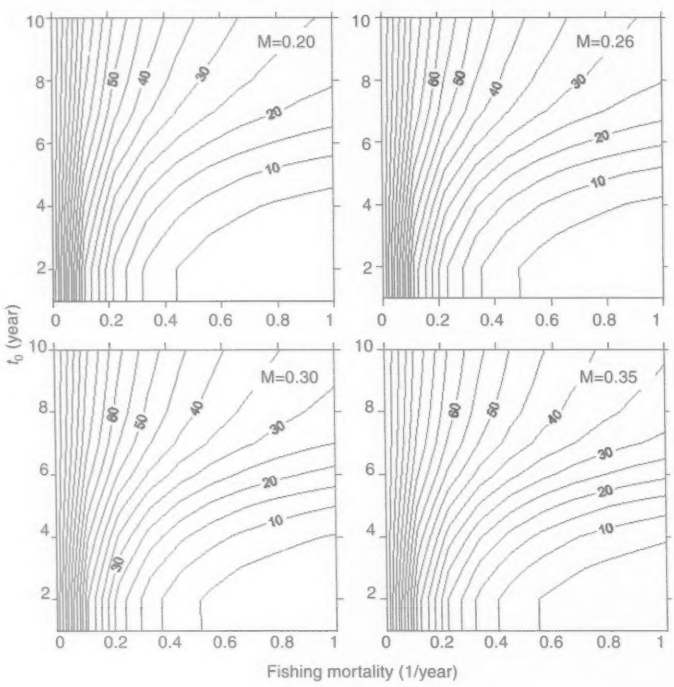


**Figure 9**

Spawning-potential ratio (*SPR*) isopleths against fishing mortality per year and age at first capture ($t_c$) at different levels of natural mortality ($M$) for female sailfish (*Istiophorus platypterus*) in waters off eastern Taiwan.

the estimate of current fishing mortality rate, the status of a fishery can be readily determined by comparing current fishing mortality with the biological reference points. Nevertheless, few results of previous studies have been discussed as to how the biological reference points based on per-recruit analyses were influenced by the uncertainties of biological parameters.

In this study, the effects of $F$, $M$, and $t_c$ on the estimates of $F_{0.1}$, $F_{SSB40}$, and $F_{SSB25}$ were evaluated by using the Monte Carlo simulation method. Although other parameters (e.g., growth) are essential inputs for per-recruit analyses, we focused on the effects of $F$, $M$, and $t_c$ which are generally more difficult to estimate owing to a lack of enough auxiliary information (Chen et al., 2007). In addition, the assumption of selectivity of fishing gear could influence the results of per-recruit analyses. Generally, longline selectivity is assumed to be asymptotic and gillnet selectivity is assumed to be dome shaped. In this study, a dome-shaped selectivity was assumed for incorporating into the per-recruit analyses. Sailfish in the waters off eastern Taiwan were exploited by various fishing gears although large proportion of the catch was made by gillnet. However, insufficient length-frequency data recorded by fishing gear lead to difficulty in estimating the selectivity for different fishing gear. Therefore, collecting the information from fishing gear for length-frequency data is necessary to evaluate the influence of different selectivity assumptions on the results of assessment.

### Implications of sex-specific assessment and management of the species

Uozumi (2003) indicated that the problems in the stock assessment of sailfish are the inability to obtain reliable biological parameters, standardization of catch per unit of effort, and a mechanism to develop reliable abundance indices. Sailfish are known to be sexually dimorphic (females grow faster and to a larger size than males (Chiang et al., 2004; Hoolihan, 2007), females become mature later than males, and sex ratio varies with length (Chiang, 2004). These attributes indicate that sex-specific assessments should be done to evaluate the status of sailfish. The sex-specific per-recruit analyses with the consideration of parameter uncertainty used in this study provide a method for evaluating management strategies.

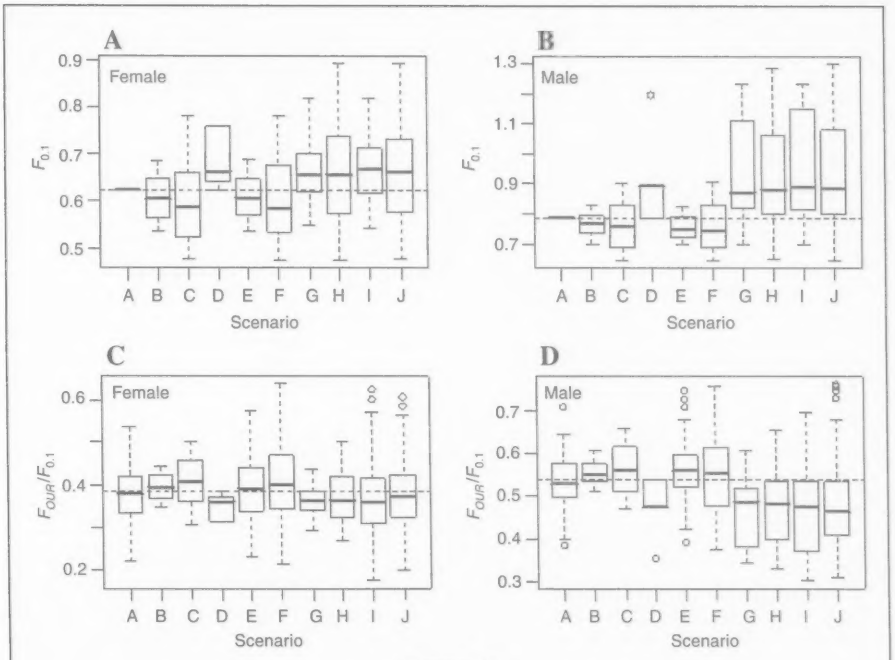


**Figure 10**

Box plots of the estimates of $F_{0.1}$ and $F_{CUR}/F_{0.1}$ for scenarios incorporating the uncertainty in total mortality ($Z$), natural mortality ($M$) and age at first capture ($t_c$) for female (**A, C**) and male (**B, D**) sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan. The center line represents the median and the boxes represent the quartiles. The whiskers extend 1.5 times the interquartile range. Open circles are outliers. Horizontal dash lines indicate the levels of reference points at base case.

There is a possibility that the low $F$ estimates may be an artifact of an influx of fish from outside the study area. Such a bias could occur if the probability that a fish will move from distant waters to within range of the eastern Taiwanese fleet is substantial and increases with the age of the fish (Sun et al., 2005). In principle, estimates for $M$ could be obtained from research (such as tagging studies to determine $M$; Hampton, 2000). However, in the short to medium term, the values for $M$ will have to be obtained from the results of studies for other stocks of sailfish. They have been similar to those obtained by Pauly's (1980) method. This difference should be evaluated in relation to environmental factors. Although recent analyses of molecular markers do not support recognition of separate Atlantic and Indo-Pacific species of sailfish (Graves and McDowell, 2003), there is no evidence to indicate that sailfish become increasingly likely to migrate to Taiwan with increasing age. Although Prince et al. (2006) examined the tagging results in the eastern Pacific Ocean, they considered this species a single stock. It is unclear whether the sailfish population in the Pacific Ocean comprises a single or multiple stocks, and their regional or global abundance is unknown (Ehrhardt and Fitchett, 2006). A tagging program in which electronic and conventional tags are used to examine the spatial movement patterns and stock structure of sailfish in this geographical region would prove beneficial for the sustainable management of the species.

There are no management measures for sailfish in the waters off eastern Taiwan at present. In the waters off eastern Taiwan, sailfish are targeted by the gillnet fishery and caught incidentally in the longline, harpoon, and set net fisheries. This makes it difficult to effectively control the fishing effort of these fisheries for sailfish. In this case, the isopleths of *SPR* indicate that increasing $t_c$ to an age between six and seven years old would likely result in modest gains in terms of *SPR* (on the order of 40%) and also hedge against recruitment overfishing. For example, the target level of 40% *SPR* could be achieved at even double values of $F_{CUR}$ if $t_c$ for female sailfish was larger than five years (the age at sexual maturity). Of course the efficacy of increasing $t_c$ would be mitigated by any substantial release mortality. At present little is known about the mortality rates of fish released from gillnet and longline vessels operating off eastern Taiwan, and further

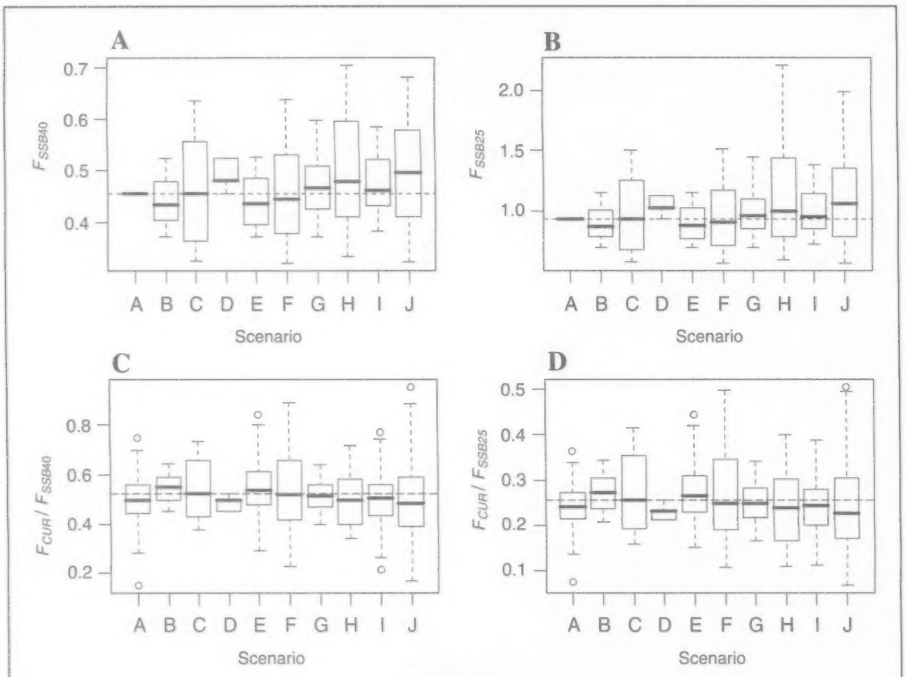


**Figure 11**

Box plots of the estimates of $F_{SSB40}$(**A**), $F_{SSB25}$(**B**), $F_{CUR}/F_{0.1SSB40}$(**C**) , and $F_{CUR}/F_{0.1SSB25}$ (**D**) for scenarios incorporating the uncertainty in total mortality ($Z$), natural mortality ($M$), and age at first capture ($t_c$) for female sailfish (*Istiophorus platypterus*) in the waters off eastern Taiwan. The center line represents the median and the box represents the quartiles. The whiskers extend 1.5 times the interquartile range. Open circles are outliers. Horizontal dashed lines indicate the levels of reference points at base case.

study is needed before we can confidently recommend increasing $t_c$ as a measure to prevent overfishing.

In summary, sex-specific per-recruit modeling coupled with Monte Carlo simulation analyses are effective in evaluating the stock status of billfish because of the sexual dimorphism and uncertainty in key life history and fishery parameters of these species. This study reveals that sailfish in the waters off eastern Taiwan appear to be moderately exploited and have relative low risk of being overfished. However, in view of the recent rapid increase in fishing effort, it is evident that the stock status and development of the fishery need to be closely monitored.

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We would like to thank two reviewers, Michael H. Prager, NMFS Southeast Fisheries Science Center, and Gerard DiNardo, NMFS Pacific Islands Fisheries Science Center, and one anonymous reviewer for their valuable and constructive comments. We thank Michael Prager for proposing the modified catch-curve method. This study was partially financially supported by the Fisheries Agency, Council of Agriculture, Taiwan, through the grant 93AS-9.1.1-FA-F1(2) to Chi-Lu Sun.

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**Abstract**—The relative value of pelagic habitat for three size classes of juvenile Pacific ocean perch (*Sebastes alutus*) was investigated by comparing their abundance and condition in two areas of the Aleutian Islands. Diet, zooplankton biomass, and water column temperatures were examined as potential factors affecting observed differences. Juvenile Pacific ocean perch abundance and condition, and zooplankton biomass varied significantly between areas, whereas juvenile Pacific ocean perch diet varied only by size class. Observed differences in fish condition may have been due to the quantity or quality of pelagic prey items consumed. For the delineation of essential demersal fish habitat, important ecological features of the pelagic habitat must therefore be considered.

# Abundance, condition, and diet of juvenile Pacific ocean perch (*Sebastes alutus*) in the Aleutian Islands

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Studies defining essential fish habitat often focus on associations of marine fishes with seafloor characteristics (Neuman and Able, 1998; La Mesa et al., 2002). Although identifying these associations is necessary to define habitats used by fishes, it is also important to explore other factors that may affect fish survival. Pelagic production of food resources, such as zooplankton, may be a factor that affects the survival of demersal fish. Bottom-up control through variation in pelagic production, may in part explain the observations of large climate-change effects on demersal fishes, including rockfishes (Hollowed and Wooster, 1992; Hollowed et al., 2001).

Pacific ocean perch (*Sebastes alutus*; POP) are an important rockfish species that migrate vertically off the seafloor and consume pelagic prey (Brodeur, 2001), thus providing a direct linkage between pelagic zooplankton and demersal fish production. In general, juvenile POP (defined as individuals <250 mm fork length [FL] based on their size at first maturity) are associated with high-relief structured habitats, such as rocky outcrops, boulder fields, deep-water sponges, and corals (Rooper and Boldt 2005; Rooper et al., 2007). Little is known of the costs and benefits to juvenile POP of residing in different habitat types or the potential linkage between pelagic prey and demersal habitats.

One measure of the relative value of different habitat types to juvenile POP may be fish condition. Fish diet, habitat, and water temperature are integrated into overall fish condition, which may affect fish growth and subsequent survival (Paul, 1997; Boldt and Haldorson, 2004). The goal of this study was to examine the relative abundance and condition of three size classes of juvenile POP in two different areas of the Aleutian Islands. Fish occupying suboptimal habitats were expected to be in poor condition compared to those living in optimal habitats. Additionally, we explored potential causes for observed differences in fish condition by examining POP diet, prey availability, and water temperature observed in the different habitats.

## Methods and materials

### Study site

Fieldwork for this study was conducted from 28 May to 9 June 2004, and from 11 August to 23 August 2004, at two

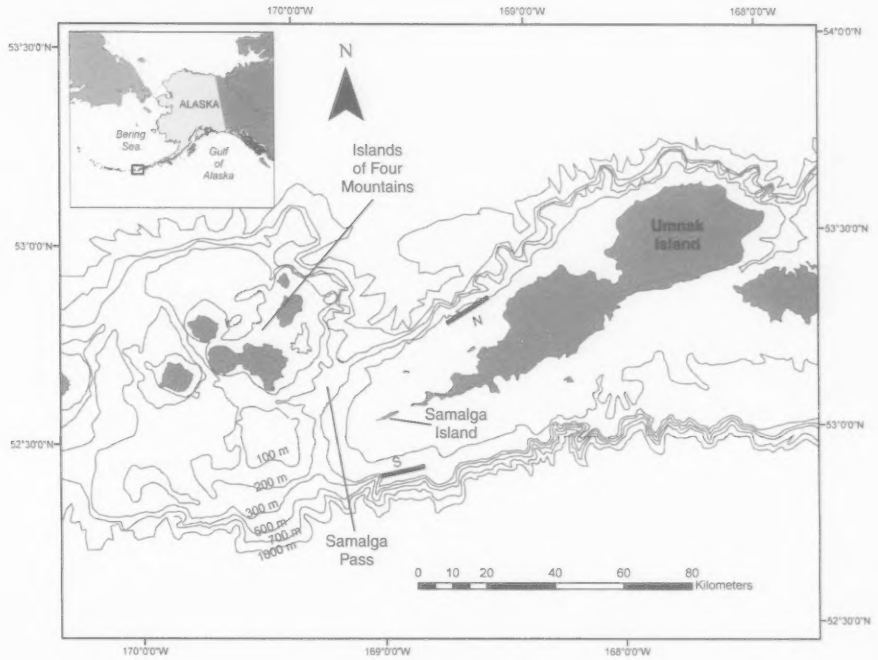


**Figure 1**

Map of the study area in the Aleutian Islands, Alaska. Study sites were located near the Islands of Four Mountains, north of Samalga Island (N), and south of Samalga Island (S). At both sites, juvenile Pacific ocean perch (*Sebastes alutus*) were collected with a bottom trawl, zooplankton were collected with a ring net, and water column temperature profiles were recorded with a conductivity, temperature, depth recorder.

sites near Samalga Pass in the Aleutian Islands (Fig. 1). One site was located on the south (S) side and one on the north (N) side of Samalga Island (Fig. 1). Two stations were sampled at each of the two sites in August and two stations were sampled at the N site in June. All the stations were located at depths from 117 to 146 m in high relief habitat (boulder fields) inhabited by juvenile POP.

### Juvenile POP abundance and size classes

Juvenile POP (<250 mm FL) were collected during daylight hours with a standard NMFS poly Nor'eastern bottom trawl. A net (with average net width of 16.5 m) was towed at a target speed of 3 knots over distances of 0.4–1.0 km. The catch per unit of effort (CPUE in number of juvenile POP per hectare) at each site was calculated by using the area swept. Juvenile POP were measured for fork length and frozen for laboratory analyses. Temperature was measured twice daily at each site with a SeaBird CTD (conductivity, temperature, depth recorder, Sea-Bird Electronics, Inc., Bellevue, WA) to obtain temperature-depth profiles.

In the laboratory, juvenile POP were thawed, measured, weighed, and dissected. Fish stomachs were weighed full and empty, and stomach contents were preserved in 10% buffered formalin for later diet analyses. Length frequencies of juvenile POP were plotted and natural breakpoints were used to divide the fish into three size categories (<160 mm FL, 160–210 mm FL, and >210 mm FL). Diet and fish condition were analyzed by these size categories and by site.

### Juvenile POP condition

Fish condition was assessed by using log-transformed length–wet-weight regression residuals (indicator of somatic growth and hereafter referred to as wet-weight residuals; $n$=226) and log-transformed length–dry-weight regression residuals (indicator of energy storage and hereafter referred to as dry-weight residuals; $n$=226). Also, the energy content of dried fish ($n$=226) was determined with a Parr 1425 Semimicro bomb calorimeter (Parr Instrument Company, Moline, IL). If available, at least 10 fish of each size category per site were dried in a 60°F drying oven or in a freeze dryer until a stable weight was reached. To test for differences in dry weights between the drying methods, thirty randomly chosen fish were individually ground and divided in half; one half of each fish was dried in the drying oven

and the other half in the freeze dryer. Also, twenty two randomly selected fish were homogenized and divided in half; one half was freeze-dried, the other half was oven-dried, and the energy content of both halves was determined with the carorimeter to determine if there were differences in energetic content attributable to the two drying methods.

### Juvenile POP diet

Whole blotted wet weights of stomach contents were recorded. Prey items were identified to a general taxonomic level (large (≥2.5 mm) copepods, small (<2.5mm) copepods, euphausiids, larvaceans, *Limacina* spp., chaetognaths, hyperiid amphipods, other), which were counted and weighed. Numerical and weight proportions of each prey group in an individual stomach were calculated and then averaged over all fish within each size category sampled at a site. The number of empty stomachs was recorded; however, empty stomachs were not included in average diet composition calculations. When the average percent number of a prey item was less than 5%, it was grouped in the "other" category. A stomach content index (SCI) was estimated as the stomach content weight as a proportion of the fish weight and averaged over all fish in each size category at each site.

### Zooplankton

In August, two zooplankton samples were collected at each of the north and south sites with a 1-m ring net (505-$\mu$ mesh) hauled vertically from depths of 40–105 m. The volume filtered ($m^3$) was estimated as the product of the area of the ring net opening and the maximum depth from which it was hauled (recorded by a temperature-depth recorder attached to the net bridle). The relative biomass of zooplankton in each study area was estimated as the settled volume of zooplankton per volume of water filtered. Zooplankton samples were preserved in 5% buffered formalin for laboratory analysis. Taxonomic components of the zooplankton samples were examined in the same manner that prey were enumerated from juvenile POP stomach contents.

### Statistical analyses

An analysis of variance (ANOVA) was used to test for differences in log-transformed juvenile POP CPUE in August between sites, with site, size class, and the interaction term as factors. An ANOVA was used to test for differences in the juvenile POP response variables: wet-weight residuals, dry-weight residuals, energy content, and percent number and weight of main prey items. Site was the factor tested for all size classes of fish sampled in August. In June, only the north site was sampled for juvenile POP; therefore comparisons with the south site were not possible for the month of June. For large fish at the north site the factors tested were month, site, and a month–site interaction term. The nonparametric Wilcoxon rank-sum test was used to determine if the SCI was significantly different between sites for each size class of juvenile POP. ANOVAs were used to test for significant differences in the numerical proportions of major taxa found in the zooplankton samples between sites in August.

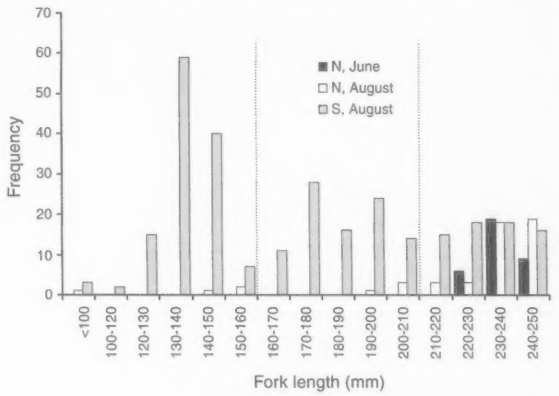


**Figure 2**

Length frequency of juvenile Pacific ocean perch (POP, *Sebastes alutus*) collected with a bottom trawl at sites north (N) and south (S) of Samalga Island in August and at the N site in June. Juvenile POP were divided into three size classes based on this length frequency (<160 mm, <210 mm, and >210 mm fork length), which correspond to ages 1–2, 3, and 4–5 years, respectively.

## Results

### Juvenile POP abundance and size classes

Juvenile POP were caught in the two trawl hauls conducted at each of the two sites in August and the two trawl hauls conducted at the north site in June. Overall catch per unit of effort (log [*CPUE*+1]) estimates indicated that the abundance of juvenile POP was significantly higher ($P$=0.027) at the south site (mean=4.22, SE=0.53) than at the north site in August (mean=1.82, SE=0.63). There were no significant differences in CPUE among size classes, and the interaction between size class and site was also insignificant. The average CPUE at the north site in June (mean=2.22, SE=1.71) was slightly higher than that measured in August at the same site.

Juvenile POP fork lengths ranged from 83 to 249 mm. Three size classes of juvenile POP were apparent in a length-frequency plot (Fig. 2): small (<160 mm FL), medium (160–210 mm FL), and large (>210 mm FL). These size categories roughly correspond to five age classes of

fish: age 1 and 2 (small), 3 (medium), and 4 and 5 years (large; D. H. Hanselman, Alaska Fisheries Science Center, personal communication). August sample sizes of small, medium, and large fish were 4, 4, and 43, respectively, at the north site, and 126, 93, and 67, respectively, at the south site. Only the large size class of juvenile POP was found in June at the north site ($n$=34).

### Juvenile POP condition

Generally, juvenile POP were in better condition at the north site than at the south site in August (Fig. 3). The linear regression of log wet weight as a function of log length was significant, as was the linear regression of log dry weight as a function of log length ($P$<0.001). There was no difference in the dry weight expressed as a function of wet weight between the drying methods; therefore, no correction factor was needed for drying technique. Both wet- and dry-weight residuals were higher at the north site for most size classes. Wet-weight residuals were significantly higher for both small and medium fish at the north site ($P$<0.05; Fig. 3). Dry-weight residuals were significantly higher for medium fish ($P$<0.001) and insignificantly higher for large fish ($P$=0.686) at the north site (Fig. 3). Dry-weight residuals for small fish appeared to be lower for fish at the north site; however, the difference was not significant ($P$=0.156; Fig. 3). Because of small sample sizes of small and medium fish at the north site, variability in estimates were high and contributed to the opposing patterns of dry- and wet-weight residuals for small fish in the north. Large fish had similar wet- and dry-weight residuals at the two locations. Large fish sampled in June had significantly lower dry-weight residuals ($P$=0.001) but similar wet-weight residuals ($P$=0.605) as those for large fish sampled in August at either site (Fig. 3).

Energy content as estimated with a calorimeter provided more precise estimates of fish condition (with lower variability) than length–weight residuals. Oven-drying resulted in a slightly lower energetic content than freeze drying (2% difference in the means between the two methods, SE=0.002). A linear relationship (slope=1.31, intercept=–1363.86, $r^2$=0.964) predicting the freeze-dried energetic content from the oven-dried energetic content was applied as a correction factor to the energetic value of the remaining oven-dried fish. Energy content varied significantly between the sites for all size classes ($P$<0.006), but not between sampling times (June and August; $P$=0.178) at the north site. Energy content of all size classes of fish was significantly higher at the north site than at the south site in August (Fig. 3). Energy content of individual juvenile POP ranged from 3,463 to 5,569 cal/g dry weight. Small fish at the south site had the lowest average energy content (4,618 cal/g dry weight), whereas, the small and medium fish at the north site had the highest average energy content (5,295 and 5,213 cal/g dry weight, respectively).

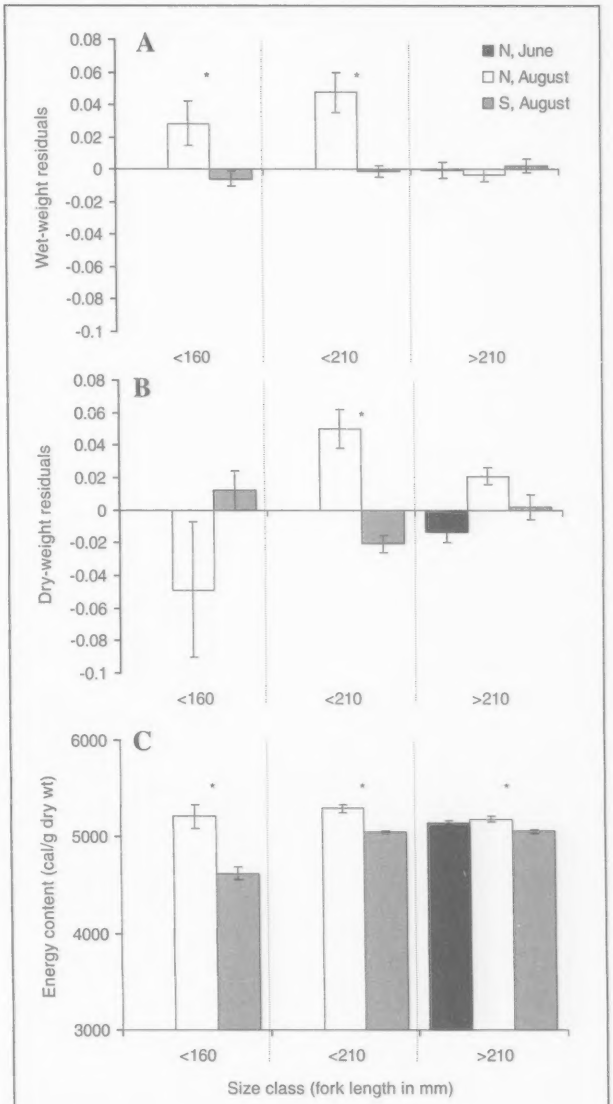


**Figure 3**

The average condition of three size classes (<160 mm, <210 mm, and >210 mm fork length) of juvenile Pacific ocean perch (*Sebastes alutus*) collected with a bottom trawl at the north (N) and south (S) Samalga Island sites in August and at the N site in June. Condition is shown three ways: (**A**) log-transformed length–wet-weight residuals; (**B**) log-transformed length–dry-weight residuals; and (**C**) whole body energy content (calories per gram dry weight). Standard error bars are shown and asterisks indicate significant differences.

### Juvenile POP diet

Generally, the SCI was highest for small fish and lowest for large fish; large fish sampled in June at the north site had significantly fuller stomachs than large fish sampled in August at the north site ($P<0.001$; Fig. 4). The SCI was similar between sites for both small and medium fish ($P=0.954$ and 0.229, respectively). In August, large fish had significantly fuller stomachs at the south site than those at the north site ($P=0.024$; Fig. 4).

The proportion of POP with empty stomachs varied among sites and months (Fig. 4). In August, the proportion of empty stomachs was higher at the north site for both medium and large POP (0.33 and 0.32, respectively). Empty stomachs were not found in small POP at the north site in August or in large POP at the north site in June (Fig. 4).

Large copepods (≥2.5 mm) comprised the majority of juvenile POP diets for all size classes; however, larger prey items such as euphausiids were consumed by medium and large juveniles (Fig. 5). Small juvenile POP (sampled in August) consumed almost exclusively large copepods, as did the large POP sampled in June. Of the small and medium POP sampled at the north and south sites in August, there were no significant differences by numerical or weight proportions in the three main prey items consumed: large copepods, euphausiids, and larvaceans ($P>0.05$). The proportion (numerical and weight) of euphausiids consumed by large POP juveniles was significantly higher at the south site ($P<0.01$). Large POP juveniles at the north site consumed significantly more large copepods in June than in August ($P<0.01$).

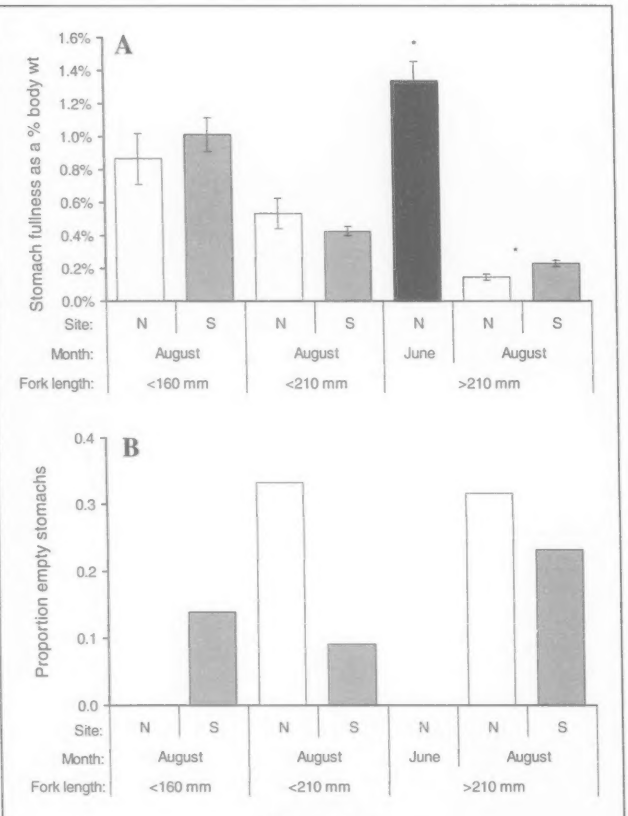


**Figure 4**

(**A**) Stomach content weight expressed as a percent of body weight (see SCI under heading "Juvenile POP diet" above), and (**B**) proportion of empty stomachs for three size classes of juvenile Pacific ocean perch (POP, *Sebastes alutus*) (<160 mm, <210 mm, and >210 mm fork length) at the north (N) and south (S) Samalga Island sites in August and at the N site in June, collected with a bottom trawl in the Aleutian Islands, Alaska. Standard error bars are shown for stomach fullness estimates. An asterisk indicates significant differences for that size class.

### Zooplankton

Zooplankton biomass, but not community composition, was significantly different between the two sites in August ($P=0.002$). Average zooplankton biomass was three times higher at the north site than at the south site (Fig. 6). Zooplankton samples at both the north and south sites were numerically dominated by large copepods. Small copepods and hyperiid amphipods were also numerically important at both sites. There were no significant differences in the proportions of large copepods, small copepods, or hyperiid amphipods between sites ($P=0.109$, 0.159, 0.365, respectively; Fig. 6). Other zooplankton components included euphausiids, chaetognaths, *Limacina* pteropods, and larvaceans. Euphausiids were more numerous at the north site than at the south site, but this difference was not significant ($P=0.792$).

## Discussion

The comparison of juvenile POP in two areas of the Aleutian Islands revealed spatial differences in the condition of fish of the same size class. Differences in fish condition have been attributed to food availability in past studies; for example, age-0 pollock with a relatively high index of condition and with high growth rates were found in areas of high prey abundance (Wilson et al., 2005). In the present study, the quantity of prey available, as indicated by the settled volume of zooplankton sampled in the water column, was very different between the sites. The ring net used in this study to sample zooplankton, however, did not sufficiently sample all juvenile POP prey; therefore, conclusions that can be based on these data are limited. In August, zooplankton biomass was high and

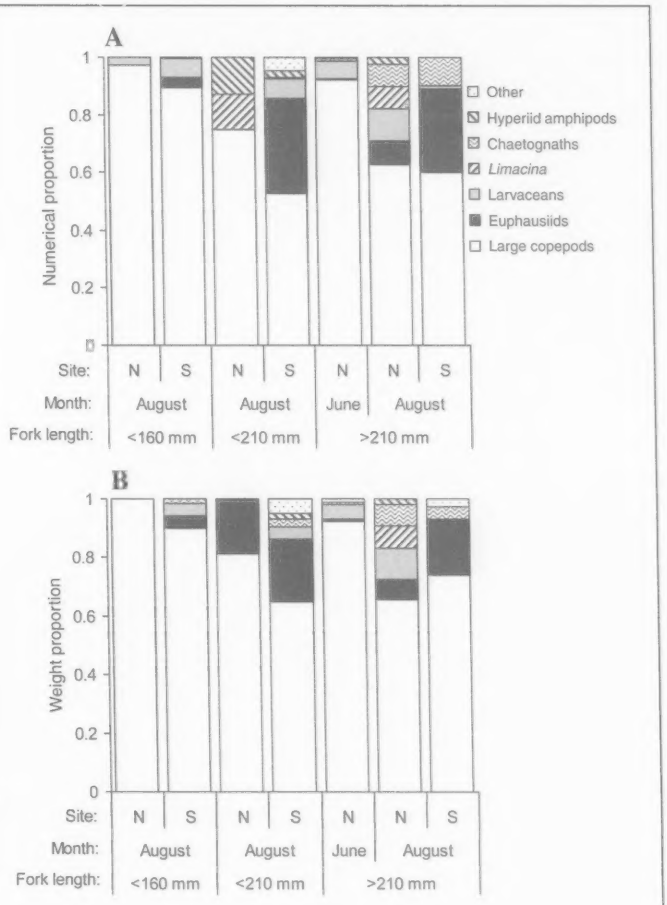


**Figure 5**

Zooplankton prey consumed by three size classes of juvenile Pacific ocean perch (*Sebastes alutus*) (<160 mm, <210 mm, and >210 mm fork length) collected with a bottom trawl at the north (N) and south (S) Samalga Island sites in August and at the north (N) site in June in the Aleutian Islands, Alaska. The average numerical (**A**) and weight (**B**) proportions of hyperiid amphipods, chaetognaths, *Limacina* pteropods, larvaceans, euphausiids, large copepods (≥2.5 mm), and other prey (<5% of prey proportions) are shown. Other prey include small copepods (<2.5 mm), barnacle cyprids, crab zoeae, fish, fish eggs, insects, larval fish, invertebrate eggs, ostracods, polychaetes, shrimp, mysids, and jellyfish.

juvenile POP abundance was low in the north, where fish condition was highest. If the large zooplankton biomass at the north site indicates enhanced prey availability to juvenile POP, it may help explain the high POP condition observed at that site.

Juvenile POP stomachs sampled in June were more than twice as full as those sampled in August at the north site, indicating that POP are able to consume more than they appeared to consume in August. This finding, along with differences in juvenile POP abundance and in zooplankton biomass between the two sample sites in August, may indicate that food limitation and competition contributed to the observed geographic differences in fish condition. In August, however, stomach fullness was similar at both sites for all size classes, indicating that fish were consuming similar amounts of food at both sites. Also, the proportion of empty stomachs tended to be higher at

the north site where fish condition was highest. Zooplankton biomass and feeding conditions before our study likely contributed to the observed differences in fish condition.

In this study the types of prey consumed by juvenile POP were very similar between the two areas, and large calanoid copepods comprised the majority of juvenile POP diets of all size classes examined. In fact, the types of prey consumed by juvenile POP varied more by size class of POP than by area. Medium and large juvenile POP consumed more larger prey, such as euphausiids, than the small juvenile POP. These findings are very similar to those found for juvenile POP in southeast Alaska (Carlson and Haight, 1976). We did not quantify juvenile POP prey to the species level; however, the type of large copepods (*Euchaeta elongata*, *Calanus marshallae*, *Neocalanus cristatus*, and *Metridia* spp.) and euphausiids (*Thysanoessa raschii*) in the zooplankton samples did not appear to differ between sites, indicating that the variety of prey species available to juvenile POP was similar between sites.

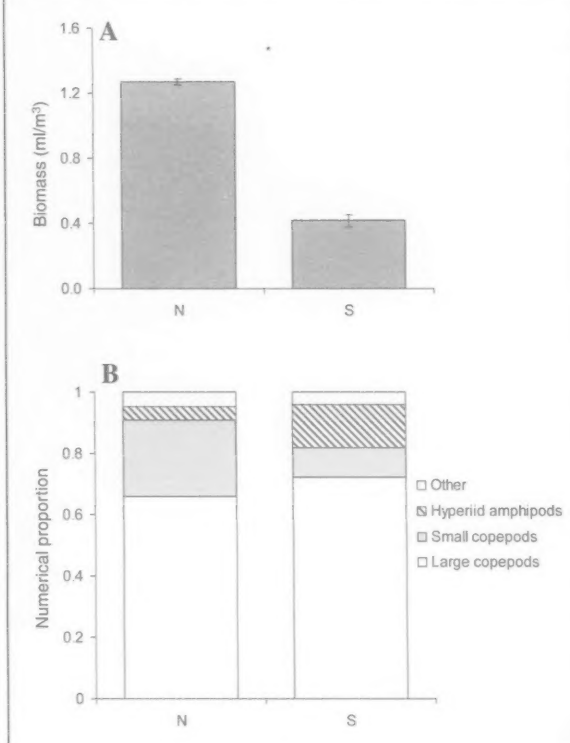


**Figure 6**

(**A**) Average zooplankton biomass (mL/m³), and (**B**) numerical proportion of main zooplankton taxa as sampled with a ring net at the north (N) and south (S) Samalga Island sites in August. Standard error bars are shown for zooplankton biomass and the asterisk indicates a significant difference. Main zooplankton taxa included hyperiid amphipods, small copepods (<2.5 mm) and large copepods (≥2.5 mm). The "other" category comprises zooplankton taxa that individually represented less than 5% of the numerical proportion of the zooplankton samples.

Water temperature and water column stratification are other factors that may affect fish condition. Bottom water temperatures were similar at both sites in August (5.4–5.6°C); however, the water column was more mixed at the north site and stratified at the south site. Water column stratification determines, in part, the amount of primary and, hence, secondary productivity by controlling nutrient flow from deeper waters. Previous studies have shown that the area north of Samalga Pass is an area of upwelling and high zooplankton biomass (Swift and Aagaard, 1976; Coyle 2005) and can be characterized as a more productive area than the south site. The higher production at the north site may lead to a higher zooplankton biomass, which we observed in this study, and higher zooplankton lipid stores and energy content, potentially benefitting predators such as juvenile POP.

In summary, we have shown that juvenile POP condition and abundance vary significantly between areas, whereas juvenile POP diet varies by size class of this species. Juvenile POP condition was higher in the area with lower juvenile POP abundance. The differences in fish condition may be due to limitation in the quantity or quality of available prey. In order to delineate essential fish habitat for demersal marine fish species, therefore, one must consider not only the presence or absence of an organism and its benthic habitat, but also the important ecological features of the pelagic habitat.

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# Modified sorting technique to mitigate the collateral mortality of trawled school prawns (*Metapenaeus macleayi*)

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**Abstract**—The potential for changes to onboard handling practices in order to improve the fate of juvenile school prawns (*Metapenaeus macleayi*) discarded during trawling were investigated in two Australian rivers (Clarence and Hunter) by comparing a purpose-built, water-filled sorting tray against a conventional dry tray across various conditions, including the range of typical delays before the start of sorting the catch (2 min vs. 15 min). Juvenile school prawns ($n$=5760), caught during 32 and 16 deployments in each river, were caged and sacrificed at four times: immediately ($T_0$), and at 24 ($T_{24}$), 72 ($T_{72}$), and 120 ($T_{120}$) hours after having been discarded. In both rivers, most mortalities occurred between $T_0$ and $T_{24}$ and, after adjusting for control deaths (<12%), were greatest for the 15-min conventional treatment (up to 41% at $T_{120}$). Mixed-effects logistic models revealed that in addition to the sampling time, method of sorting, and delay in sorting, the weight of the catch, salinity, and percentage cloud cover were significant predictors of mortality. Although trawling caused some mortalities and comparable stress (measured as $_L$-lactate) in all school prawns, use of the water tray lessened the negative impacts of some of the above factors across both the 2-min and 15-min delays in sorting so that the overall discard mortality was reduced by more than a third. When used in conjunction with selective trawls, widespread application of the water tray should help to improve the sustainability of trawling for school prawns.

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Mitigating the collateral mortality from trawl fisheries is a complex issue that requires multifaceted strategies (Davis, 2002; Broadhurst et al., 2006). One option is to spatially and temporally restrict fishing to avoid known large assemblages of nontarget organisms (termed "bycatch") (Andrew and Pepperell, 1992). A broader approach involves physical modifications to gears to improve their selection of species and size of species. Such modifications are assumed to indirectly reduce mortality on the premise that the survival of escapees during fishing is generally much greater than those of organisms brought to the surface and discarded (Broadhurst et al., 2006). However, few (if any) modifications to trawls are 100% effective and therefore, despite their use in many fisheries, there remains at least some unwanted bycatch (Andrew and Pepperell, 1992; Broadhurst, 2000). A remaining ancillary strategy that has been rarely applied, but which may help to address this problem, is to refine operational and postcapture handling techniques to improve the survival of discarded bycatch (e.g., Gamito and Cabral, 2003; Macbeth et al., 2006).

Similar to attempts in many of the world's penaeid fisheries, attempts at resolving collateral mortality in the New South Wales river prawn-trawl fishery have mostly focused on the first two strategies described above. From the early 1940s until 2003, this fishery comprised over 300 small (mostly <10-m) trawlers predominantly targeting either school (*Metapenaeus macleayi*) or eastern king (*Penaeus plebejus*) prawns in five rivers and estuaries. In addition to the targeted catches, these trawlers have traditionally caught, and then discarded, large quantities of bycatch (estimated at up to 177 t/yr in some rivers and estuaries; Liggins and Kennelly, 1996; Liggins et al., 1996), often comprising juveniles of economically important species, including penaeids too small for sale (<approximately 15–17 mm carapace length [CL]). During the last 20 years, concerns over the mortality of these organisms have culminated in complete closures to fishing at some locations, and the development of, and legislation for, modifications to trawls, including bycatch reduction devices (BRDs) and square-mesh codends, for use throughout the remaining fleet (Broadhurst and Kennelly, 1996; Macbeth et al., 2007).

Effort in this fishery has now been reduced to 204 vessels distributed among three rivers; the Clarence (114 vessels), Hawkesbury (61) and Hunter (29). Although the modified gears used by the operators of these vessels are more selective than histori-

cal configurations, even under optimal conditions the relative reductions of many bycatch individuals are only between approximately 50% and 70% and therefore, at times, large numbers are still caught and discarded (Broadhurst and Kennelly, 1996). Recent work with trawlers in the Clarence River indicates that simply deploying trawls for shorter durations (30 rather than 60 min) and sorting catches in water can minimize the negative impacts to some of these discards (Uhlmann and Broadhurst, 2007; Broadhurst et al., 2008).

In particular, Broadhurst et al. (2008) described a purpose-built, onboard water-sorting system (termed a "water tray") designed to facilitate the separation of prawns and fish, and then prawns into retained and discarded categories, while minimizing their exposure to air. This system was examined for its utility in reducing the mortality of fish after both immediate and delayed discarding from trawls deployed for the shortest commercially viable period (30 min). Although the mechanical interactions associated with trawling meant that the mortalities of many fish remained high, there were significant reductions in fatalities when the water tray was used during longer delays in starting sorting.

No work has been done to assess whether the water tray similarly reduces the mortality of discarded juvenile prawns, although two relevant pilot studies support the application of this modification (Macbeth et al., 2006; Broadhurst and Uhlmann, 2007). Specifically, during four deployments at one location in the Clarence River, Macbeth et al. (2006) observed that the short-term (three days) mortality of juvenile school prawns was reduced from approximately 35% during conventional sorting to about 16%, simply by holding them in water-filled containers. However, irrespective of their handling, all surviving school prawns showed similar elevated stress responses (measured as $_{L}$-lactate) over the monitoring period, which may have increased their susceptibility to other types of mortality (e.g., through infection or predation). By contrast, using comparable replication, Broadhurst and Uhlmann (2007) observed that regardless of their handling (including maximum and minimum gear deployments and subsequent air exposure), school prawns appeared resilient to both seine- and trawl-induced impacts, which manifested as total mortalities of <15%. The lack of impacts was further reflected by a return of elevated $_{L}$-lactate concentrations immediately to baseline levels within 24 hours after the prawns had been discarded.

At least some of the observed discrepancies between the two studies above probably reflect their limited replication in space (one location) and time (one day of fishing). Other studies have demonstrated that a range of technical (e.g., gear design, deployment duration, and speed), biological (e.g., species, physiology, size, and catch volume and composition) and environmental (e.g., temperature, hypoxia, sea state, and light) factors can have complex interacting effects on the fate of discards during trawling (Davis, 2002; Broadhurst et al., 2006). It seems appropriate, therefore, that during any study that seeks to assess the utility of modifications to reduce collateral mortality, adequate information is collected on these factors across a range of commercial conditions. We sought to use this approach to assess the water tray for reducing the mortality of discarded school prawns in the Clarence and Hunter rivers.

## Materials and methods

### Trawlers and the water tray

Separate experiments were undertaken in the Clarence River (29°27′S, 153°09′E) between March and April 2007 and in the Hunter River (32°53′S, 151°45′E) between February and April 2008. A commercial prawn trawler (<14 m in length) rigged with standard twin trawls was used in the Clarence River and a commercial prawn trawler (<14 m) rigged with a single trawl was used in the Hunter River. All trawls were attached to square-mesh codends (27-mm mesh). The Hunter River trawler was equipped with a large horizontal canvas awning that covered most of the back deck (and catch sorting area). The Clarence River trawler had a much smaller, obliquely orientated, and less effective cover made from loose-weave polyvinyl chloride (PVC).

Before starting the experiments, the conventional sorting trays were removed from both vessels and replaced with a modified design (comparable in price to conventional trays), termed the "water tray" which was scaled to fit, and measured 1800×1500×300 mm (Clarence River trawler) and 1200×1190×300 mm (Hunter River trawler) (Fig. 1 and Broadhurst et al., 2008). Irrespective of overall dimensions, the design of the water tray remained similar and included a V-shaped bottom, designed to concentrate the catch towards its center and below a vertically orientated, variable opening (Fig. 1). A pump provided flow-through water from the river at 30 L/min to the water tray through a 50-mm diameter inlet located in one side. For the Clarence River trawler, a metal screen (50-mm mesh size) was positioned horizontally above the bottom of the water tray to allow school prawns to filter through onto the V-shaped bottom, separating them from any larger fish (Fig. 1). This metal screen was removed from the water tray that was used on the Hunter River trawler. During the sorting process in each water tray, nonpenaeid bycatch was either removed by hand, or directed out of the variable opening along with water and collected in a 35-L container. Once the bycatch was removed, school prawns were washed out with the remaining water and passed over a riddler (made from hexagonal mesh; Fig. 1), which separated unwanted and retained individuals into two 35-L containers (see also Macbeth et al., 2006 for details).

### Monitoring sites

Two 3000-L polyethylene holding tanks were positioned at one location on the banks of each river. All tanks

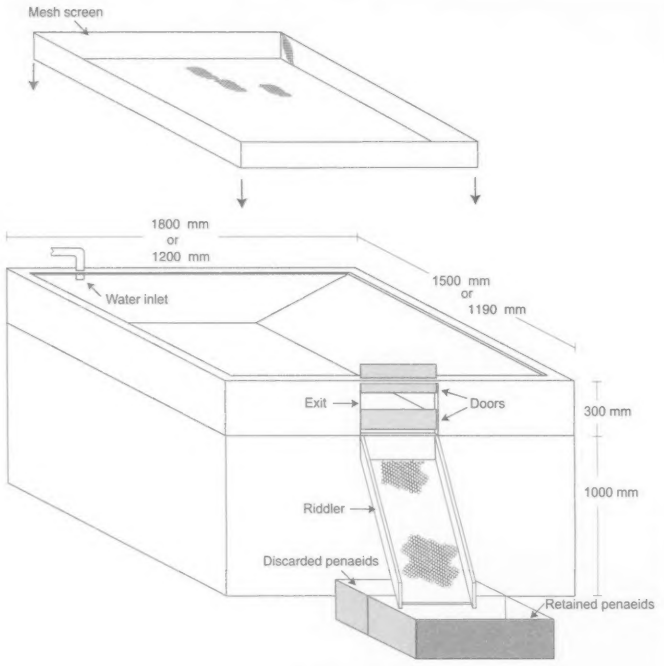


**Figure 1**

Schematic diagram of the water tray and mechanism by which school prawns (*Metapenaeus macleayi*) were riddled into discarded and retained categories in the Clarence and Hunter rivers during 2007 and 2008.

were supplied with local flow-through seawater (at a maximum rate of 63 L/min) and aerated by stone diffusers. At two sites (termed "monitoring sites") within the limits of trawling in each river, several 10-mm diameter ropes (50 m in length) were attached horizontally at a level corresponding with the average low tide to 2.5 m stanchions or existing pylons (fixed to the river bed). The rope configurations were designed to secure up to 90 portable cylindrical cages (0.3-m diameter × 0.4-m depth), each made from a modified 35-L bucket and comprising one top and three lateral openings that were covered by 6-mm PVC mesh (each <230 $cm^2$) and a solid base filled with locally collected sediment to a depth of about 6 cm (see also Broadhurst and Uhlmann, 2007 for details). Clips were attached to the tops of each cage so that they could be suspended along the 10-mm diameter rope. The cages were designed so that two could fit into in 75-L aerated water-filled PVC containers located on a dory, enabling them to be transported between the fishing and monitoring sites.

### Experimental design

In both rivers, between seven and five days before starting each experiment, approximately 1000 school prawns were collected in <5-min trawls rigged with a fine-meshed knotless polyamide, 10-mm mesh codend towed slowly in shallow water. At the end of each deployment, the codend was emptied into a water-filled container. Live and active juvenile school prawns were quickly removed, placed in tanks supplied with oxygen and transported to the two holding tanks on the river bank. The captive school prawns were fed chopped pilchard (*Sardinops neopilchardus*) at a rate of 5% biomass/24 hr and left to recover for at least five days, after which surviving, intact individuals were used as controls in the experiments described below.

Four treatment groups associated with trawling and discarding and one control group were examined in each river. The four treatments were chosen to represent the temporal limits of the conventional (i.e., dry tray) and modified (i.e., water tray) onboard handling of catches. All treatments comprised a 30-min deployment of the trawl, followed by the sorting of unwanted school prawns that was started after one of the following treatments: 1) a 2-min delay in a dry tray (termed the "2-min conventional-tray" treatment); 2) a 2-min delay in the water tray ("2-min water-tray" treatment); 3) a 15-min delay in the dry tray ("15-min conventional-tray" treatment); or 4) a 15-min delay in the water tray

("15-min water-tray" treatment). The conventional-tray treatments involved leaving the water tray empty of water and removing the mesh-separating screen. The 35-L container for the riddled, unwanted school prawns was filled with water during the water-tray treatments but was left dry for the conventional-tray treatments. Two replicates of each treatment were completed on each of four days of fishing in the Clarence River and two days of fishing in the Hunter River.

For each treatment, immediately after the unwanted school prawns were separated by the riddler into either a dry (conventional-tray treatments) or water-filled (water-tray treatments) 35-L container (Fig. 1), the transport dory was positioned alongside the trawler and 120 individuals were randomly selected and "discarded" into groups of 10 into 12 cages submerged in the water-filled 75-L PVC containers. Three of these cages were sampled immediately (termed $T_0$—see below), before the remaining nine were transported to the closest monitoring site and attached to the 10-mm rope within 20 min, and without exposing any of the school prawns to air. During transfer, all caged school prawns were held in aerated water and the water quality was checked with an Horiba U10 meter (Horiba, Irvine, CA) and maintained (via exchange) at the same levels as that recorded at the surface of the deployment site.

Within six hours of the first treatment deployment, 240 school prawns were removed from the holding tanks by scoop nets and placed in groups of 10 into 24 cages submerged in the water-filled 75-L PVC containers onboard the dory (i.e., the same number of prawns as that for the two replicate deployments of each treatment). Six cages were sampled immediately ($T_0$—see below), while the remaining 18 cages were transferred to the same monitoring sites as those housing the treatment school prawns and used as balanced controls in each experiment. For each replicate of the control and treatment groups on each day of fishing, school prawns in three of the cages were sacrificed and sampled at three times: 24 ($T_{24}$), 72 ($T_{72}$), and 120 ($T_{120}$) hours after $T_0$.

### Data collected

The following data were collected during each deployment in both experiments: towing speed (m/s); fishing depth (m); duration of air exposure of the catch (min); air temperature (°C); percent cloud cover; and the numbers and weights (kg) of retained and discarded catches. The Horiba U10 was used to record replicate measures of water temperature (°C), dissolved oxygen (mg/L), and salinity (psu) in the water tray. An EC 350 Greenspan Smart Sensor (Tyco Environmental Systems, Lakewood, NJ) was attached to the trawl to provide replicate measures of the conductivity (μS/cm) and the temperature of the river once each minute during each deployment (except on the first day of fishing in the Clarence River). An algorithm was used to convert the normalized conductivity readings to salinities. Means of these readings were used to provide a datum for the salinity and for the temperature at the surface and bottom for each deployment.

At each sampling time, the three cages from the two replicates of the control and each of the four treatment groups from each day of fishing were removed from the monitoring site. The sediments from the cages were emptied onto a tray. The numbers of alive and dead school prawns were recorded, and, if possible, they were measured to the nearest 1-mm CL. During the Hunter River experiment, the binary molt status (hard or soft) of school prawns was also noted by the rigidity of their carapace. This was not done during the Clarence River experiment because all school prawns were clearly intermolts (i.e., hard). For one randomly selected fishing day during the Clarence River experiment, two live school prawns were immediately selected from two of the cages for each replicate of the treatment and control groups at the $T_0$, $T_{72}$, and $T_{120}$ sampling times and secured in aluminum satchels before being placed in liquid nitrogen. These frozen samples were later analyzed for $_L$-lactate (μmol/g) to provide an indication of the severity of anaerobic stress and subsequent recovery, by following the methods described by Broadhurst et al. (2002).

### Statistical analyses

The data collected from each experiment were analyzed separately. Appropriate environmental, technical, and biological data collected from the gear deployments were treated as either fixed, categorical, or continuous variables. Where there was sufficient replication, these variables were considered with the fixed factors of primary interest: "method of sorting" (conventional vs. water tray); delay in sorting" (2 min vs. 15 min); and sampling time ($T_0$, $T_{24}$, $T_{72}$ or $T_{120}$); and the random factors of fishing days, deployments, and cages in mixed-effects logistic models were fitted to the dichotomous status (dead vs. alive) of the trawled-and-discarded caged school prawns.

For each experiment, three separate models were used for trawled-and-discarded school prawns and comprised data from 1) all deployments, 2) the water-tray deployments, and 3) the conventional-tray deployments only. A fourth model was fitted to control, caged school prawns, and was restricted to the above random factors and the fixed effect of sampling time. All models were fitted by using the lmer function in the R statistical software (The R Foundation for Statistical Computing, Vienna, Austria). A stepwise variable search algorithm was employed with the most parsimonious fit based on the lowest Akaike's information criterion. The total mortalities of school prawns subjected to the four handling treatments of interest were eventually adjusted for deaths to the controls.

A balanced four-factor analysis of variance (ANOVA) was used to examine differences among treatment and control groups for the levels of $_L$-lactate (μmol/g) in school prawns from one fishing day in the Clarence River experiment. The model used the following fac-

**Table 1**

Summary of mean (±standard deviation) key technical, environmental, and biological variables collected during the deployments of trawls to assess the discard mortality of school prawns (*Metapenaeus macleayi*) in the Clarence and Hunter rivers during 2007 and 2008. The number of replicate deployments is in parenthesis.

| Variable | Clarence River | Hunter River |
|---|---|---|
| Technical | | |
| Deployment | | |
| Speed (m/s) | 1.4 ±0.2 (32) | 1.3 ±0.1 (16) |
| Depth (m) | 7.3 ±1.8 (32) | 6.3 ±1.7 (16) |
| Environmental | | |
| River temperature (°C) | | |
| Surface | 23.9 ±1.1 (32) | 22.5 ±0.8 (16) |
| Bottom | 23.8 ±1.1 (24) | 22.8 ±0.3 (16) |
| River salinity (psu) | | |
| Surface | 3.7 ±2.3 (32) | 4.3 ±1.8 (16) |
| Bottom | 5.4 ±1.9 (24) | 10.2 ±5.8 (16) |
| Cloud cover (%) | 42.9 ±38.8 (32) | 3.8 ±6.2 (16) |
| Air temperature (°C) | 22.7 ±3.3 (32) | 22.6 ±4.5 (16) |
| Water tray | | |
| Dissolved oxygen (mg/L) | 5.8 ±1.0 (16) | 3.8 ±0.8 (8) |
| Temperature (°C) | 24.4 ±1.6 (16) | 22.0 ±1.6 (8) |
| Salinity (psu) | 2.5 ±1.5 (16) | 4.6 ±2.3 (8) |
| Catch air exposure (min) | | |
| 2-min conventional tray | 13.8 ±5.1 (8) | 6.3 ±1.9 (4) |
| 15-min conventional tray | 23.5 ±6.0 (8) | 20.0 ±2.7 (4) |
| 2-min water tray | 2.0 ±1.0 (8) | 1.0 ±0.0 (4) |
| 15-min water tray | 1.6 ±0.7 (8) | 1.3 ±0.5 (4) |
| Biological | | |
| Weight of catch (kg) | | |
| Total | 11.8 ±7.4 (32) | 19.4 ±16.1 (16) |
| School prawns | 9.7 ±6.6 (32) | 18.0 ±15.7 (16) |
| Carapace length (mm) of trawled-and-monitored school prawns: | 15.3 ±2.6 (32) | 15.1 ±3.8 (16) |

tors: treatment of prawns (four trawling and one control group); sample times ($T_0$, $T_{24}$, $T_{120}$ and $T_{120}$); deployments ($n$=2); and cages ($n$=3). The factors "sampling time" and "treatment of prawns" were considered orthogonal to each other and fixed. Deployments were random and nested in the treatment of prawns, whereas cages were nested in all factors. Data were transformed as required and tested for homogeneity of variances by using Cochran's test. Any missing replicates were replaced with the cell mean and the residual degrees of freedom were adjusted accordingly. Significant $F$-ratios of interest were examined with Student-Newman-Keuls multiple comparisons of means tests.

## Results

School prawns represented more than 82% of the total catch from the Clarence River and 93% of the total catch from the Hunter River, and although their mean catches per 30-min deployment were quite variable, were still within the ranges typically experienced in the fishery (Table 1). Bycatches were comparatively low in both experiments and, in addition to juvenile school prawns (approximately 9% of the total school prawn catch in each river), mostly comprised fish <200 mm total length, such as yellowfin bream (*Acanthopagrus australis*), southern herring (*Herklotsichthys castelnaui*), silver biddy (*Gerres subfasciatus*), and narrow-banded sole (*Aseraggodes macleayanus*).

Many of the technical and environmental variables were comparable between experiments and except for salinity and cloud cover, remained fairly consistent among replicate deployments (Table 1). In both rivers, there was evidence of a salinity gradient between the surface and bottom (Table 1). Catches were subjected to similar mean durations of air exposure during the 15-min conventional-tray (>20 min) and all water-tray (<2 min) treatments during both experiments. By contrast, and although highly variable, the mean duration of air exposure for catches handled during the 2-min conventional-tray treatment in the Clarence

River (approximately 14 minutes) was greater than that in the Hunter River (approximately 6 minutes; Table 1).

The first mixed-effects model that was applied to all of the data for the trawled-and-discarded school prawns in both experiments included the fixed factors of sorting method (conventional vs. water tray), delay in sorting (2 minutes vs. 15 minutes), and their interaction, sample time, and the weight of total catch, and salinity at the bottom of the river during fishing. The second and third models were applied to the water-tray and conventional-tray deployments. Both models included sorting method (conventional vs. water tray), delay in sorting (2 minutes vs. 15 minutes), and their interaction, sampling time, and the weight of total catch. Water tray salinity and dissolved oxygen were also included in the second model, and air temperature and cloud cover were used in the third model. The fourth model was applied to the control data with sampling time as the only the fixed effect. The stage of molt was not included in any of the models for the Hunter River data because it was not possible to quantify the condition of all deceased individuals. In any case, only 13.0% of all school prawns sampled at $T_0$ had soft exoskeletons. Similarly, it was not possible to measure the CL of all dead school prawns at $T_{24}$, $T_{72}$, and $T_{120}$, and therefore size could not be considered in the analyses.

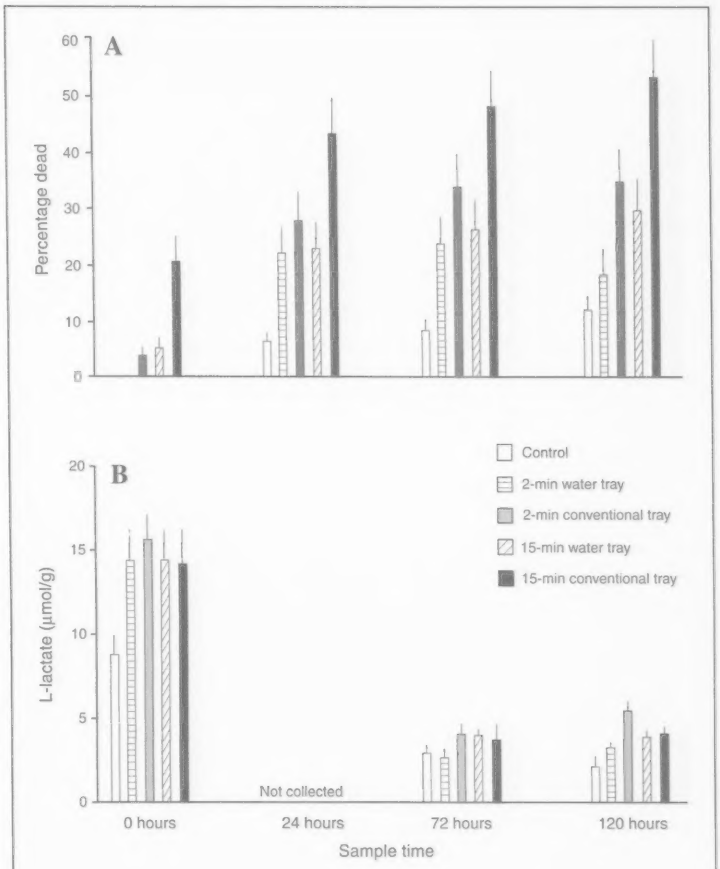


**Figure 2**

Differences in mean (± standard error) for (**A**) predicted percent mortality and for (**B**) concentrations of $_L$-lactate for school prawns (*Metapenaeus macleayi*) between the onboard sorting treatments and control for each of the relevant sample times in the Clarence River during 2007. No data were collected for $_L$-lactate at $T_{24}$.

### Clarence River experiment

A total of 4800 school prawns were caged during the experiment, of which 240 individuals were in each of the control and four treatment groups at each of the four sampling times. Seventy school prawns escaped (as a result of faulty lids on their cages) before being sampled; they escaped from one cage for the 15-min conventional-tray treatment at each of $T_{24}$ and $T_{72}$, two cages for the 15-min conventional-tray treatment at $T_{120}$, one cage for the 2-min water-tray treatment at $T_{24}$, and two cages of controls at $T_{72}$. Up to 12.0% of the control school prawns were dead at each sampling time (attributed to handling during transfer, being caged, or natural causes, Fig. 2A), providing adjusted temporal mortalities of up to 41.4% and 18.2% for the 15-min conventional and water-tray treatments, respectively (Table 2).

Irrespective of the four mixed-effects models fitted to the various data sets, or the treatment of school prawns, there was a strong significant impact of sampling time on mortality that was largely attributable to the death of most individuals during their first 24 hours of caging, after which the rate of attrition stabilized (Tables 2 and 3 , Fig. 2A , $P<0.01$). All three analyses of the trawled-and-discarded school prawns also showed a significant impact of the delay in the start of sorting, with an overall predicted (and unadjusted) mean ±standard error (SE) of 31.6 ±2.4% after 15 min, compared to only 21.2 ±1.9% after 2 min (Table 2, $P<0.05$).

In addition to the main effects of delay in sorting and sampling time, sorting method had a significant impact across all deployments, and there were consistently greater mortalities to those individuals discarded after sorting in the conventional tray (predicted unadjusted mean of 33.5 ±2.4%) than in the water tray (19.3 ±1.8%), and especially after a 15-min delay (predicted

unadjusted mean ±SE of up to 53.4 ±6.5%; Tables 2 and 3, Fig. 2A, $P<0.05$). This clear trend in deaths precluded any interaction between sorting method and delay, although these means are presented in Fig. 2A for ease of interpretation (Table 3, $P>0.1$). The first model also detected a significant negative relationship between mortality and salinity at the bottom of the river (Table 3, $P<0.01$). The same significant negative relationship with mortality was observed for salinity in the water tray in the second model (Table 3, $P<0.01$). The third model identified total catch weight as having a significant positive relationship with the mortality of conventionally handled school prawns, whereas cloud cover had a negative relationship (Table 3, $P<0.05$).

ANOVA returned significant $F$-ratios for the treatment of prawns and sampling times for the levels of $_L$-lactate in surviving school prawns from one randomly selected fishing day (Table 4 , $P<0.01$). There was no interaction between these factors ($P>0.05$), but the corresponding means are presented for clarity (Table 4, Fig. 2B). Student-Newman-Keuls tests revealed that the overall mean ±SE concentration of $_L$-lactate in control school prawns (4.63 ±0.76 μmol/g) was significantly lower than all other treatments; which remained similar at between 6.77 ±1.28 and 8.40 ±1.21 μmol/g ($P<0.05$). Irrespective of the treatment of school prawns, all prawns had significantly greater concentrations of $_L$-lactate at $T_0$ (13.49 ±0.81 μmol/g) than at $T_{72}$ (3.48 ±0.28 μmol/g) and $T_{120}$ (3.78 ±0.27 μmol/g) (Fig. 2B, $P<0.05$).

**Table 2**

Percentage of total mortalities of trawled-and-discarded school prawns (*Metapenaeus macleayi*) adjusted for deaths to the controls for the sorting treatments of interest (2-min and 15-min delays in the conventional and water trays) at the four sampling times (immediately [$T_0$], and after 24 hr [$T_{24}$], 72 hr [$T_{72}$], and 120 hr [$T_{120}$]) in the Clarence and Hunter rivers during 2007 and 2008.

| | Conventional tray | | Water tray | |
|---|---|---|---|---|
| | 2-min delay | 15-min delay | 2-min delay | 15-min delay |
| Clarence River | | | | |
| $T_0$ | 3.8 | 20.7 | 0.0 | 5.2 |
| $T_{24}$ | 21.4 | 37.0 | 15.7 | 16.6 |
| $T_{72}$ | 25.8 | 40.0 | 15.5 | 18.2 |
| $T_{120}$ | 22.8 | 41.4 | 6.3 | 17.8 |
| Hunter River | | | | |
| $T_0$ | 5.7 | 15.7 | 0.0 | 3.0 |
| $T_{24}$ | 29.8 | 29.0 | 13.6 | 18.1 |
| $T_{72}$ | 26.4 | 35.7 | 20.2 | 21.5 |
| $T_{120}$ | 20.6 | 34.8 | 18.6 | 8.9 |

## Hunter River experiment

A total of 2400 school prawns were caged across the control and four treatment groups; of which 10 (in one cage from the 15-min conventional-tray treatment) escaped prior to their designated sampling time ($T_{120}$). Similar to the Clarence River experiment, control deaths ranged between predicted means of 0.0% and 11.7%, providing adjusted total mortalities of up to 35.7% and 21.5% for the 15-min conventional- and water-tray treatments, respectively (Table 2, Fig. 3).

**Table 3**

Summary of variables tested in mixed-effects logistic models for their independence of the mortality of trawled-and-discarded school prawns (*Metapenaeus macleayi*) in the Clarence and Hunter rivers during 2007 and 2008. Four separate models were applied: the first to all of the available data for trawled-and-discarded individuals (All); the second and third to only those discarded from the water tray (WT) and conventional tray (CT) treatments, respectively; and the fourth to the data for the controls (C). ○ $P>0.1$; ● $P<0.1$; *$P<0.05$; **$P<0.01$; --, term not considered in the model.

| | Clarence River | | | | Hunter River | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | All | WT | CT | C | All | WT | CT | C |
| Sampling time | ** | ** | ** | ** | ** | ** | ** | ** |
| Sorting method (M) | ** | -- | -- | -- | ** | -- | -- | -- |
| Sorting delay (S) | ** | * | ** | -- | ○ | ○ | ** | -- |
| $M \times S$ | ○ | -- | -- | -- | ○ | -- | -- | -- |
| Total weight of catch | ● | ○ | ** | -- | ** | ** | ○ | -- |
| Salinity | | | | | | | | |
| bottom | ** | -- | ○ | -- | ○ | ○ | ○ | -- |
| surface (water tray) | -- | ** | -- | -- | -- | ○ | -- | -- |
| Dissolved oxygen (water tray) | -- | ○ | -- | -- | -- | ○ | -- | -- |
| Air temperature | -- | -- | ○ | -- | -- | -- | ○ | -- |
| Cloud cover | -- | -- | * | -- | -- | -- | ○ | -- |

As with the Clarence River analyses, there was a significant effect of sampling time detected in all four mixed-effects models that was largely due to proportionally more deaths to school prawns during the first 24 hours, irrespective of their treatment (Table 3, Fig. 3, $P<0.01$). The method of sorting was also significant in the first model applied across all treatment deployments, with relatively greater deaths after discarding from the conventional tray (overall unadjusted predicted mean ±SE of 29.3 ±2.0%), than from the water tray (17.6 ±1.6%) (Table 3, Fig. 3, $P<0.01$). Sorting delay had no impact on the mortality of school prawns discarded across all deployments, or from the water tray, but was significant for those that were conventionally sorted (mortalities of 25.8 ±2.5% for the 2-min delay, and 32.9 ±2.8% for the 15-min delay; Table 3, $P<0.01$).

The only remaining significant main effect in any of the models was the total weight of catch, identified as having a positive relationship with mortality across all deployments, and those restricted to sorting in the water tray (Table 3, $P<0.01$). For the water-tray deployments, individuals caught during the largest catch weights (47 kg) were more than 2.5 times as likely to be dead at each sampling time than those from the smallest catches (4 kg, Fig. 4). For both the $T_{72}$ and $T_{120}$ sampling times, the absolute probabilities of mortality during the largest and smallest catch weights were approximately 43% vs. 16% (Fig. 4).

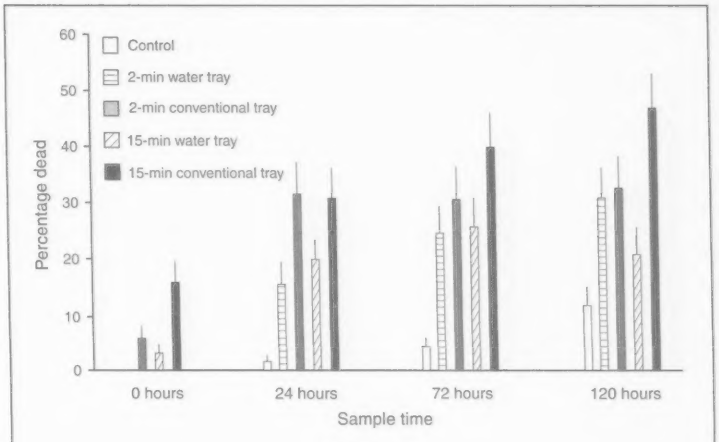


**Figure 3**

Differences in mean (± standard error) predicted percent mortality of school prawns (*Metapenaeus macleayi*) between the onboard sorting treatments and the controls for each of the sampling times in the Hunter River during 2008.

**Table 4**

Summary of model terms, *F*-ratios, and degrees of freedom (df) for ANOVA used to investigate $_L$-lactate among trawled-and-discarded (2- and 15-min conventional- and water-tray treatments) and control school prawns (*Metapenaeus macleayi*) in the Clarence River during one day of fishing in 2007. Data were ln($x$+1) transformed. Three replicates were missing, and therefore the cell means were used and the df was adjusted accordingly. **$P<0.01$.

| Variable | *F*-ratio | df |
|---|---|---|
| Treatment of prawns | 14.09** | 4, 5 |
| Deployments (treatment of prawns) | 0.58 | 5, 30 |
| Sampling times | 93.41** | 2, 10 |
| Treatment of prawns × sampling times | 0.73 | 8, 10 |
| Sampling times × deployment (treatment of prawns) | 1.55 | 10, 30 |
| Cages (treatment of prawns × sampling times × deployments) | 0.95 | 30, 57 |

## Discussion

The mortalities of school prawns after being trawled, conventionally discarded, and adjusted for control fatalities were consistent between experiments and encompassed the range of earlier estimates by Macbeth et al. (2006). Further, the unadjusted mortalities were comparable to those recorded for several other similarly treated decapods monitored without controls, including caridians (1–30%, Wassenberg and Hill, 1989; Cabral et al., 2002), brachyurans (0–50%, Hill and Wassenberg, 1990; Wassenberg and Hill, 1993; Kaiser and Spencer, 1995) and anomurans (0–19%, Kaiser and Spencer, 1995). These rates of death are considerably lower than those typically observed for many trawled-and-discarded fish and mollusks and further support the resilience of school prawns, and crustaceans in general, for withstanding a range of trawl-induced impacts (Broadhurst et al., 2006).

Irrespective of apparent broad phyla-specific differences among collateral trawl mortalities, as is the case for several locally caught fish (Broadhurst et al., 2008), use of the water tray significantly reduced the short-term mortality of school prawns in both experiments. The extent of these reductions can be explained by attempting to partition mortality, along with the various significant predictors, into those fatalities caused 1) by the trawling process, and 2) after onboard handling.

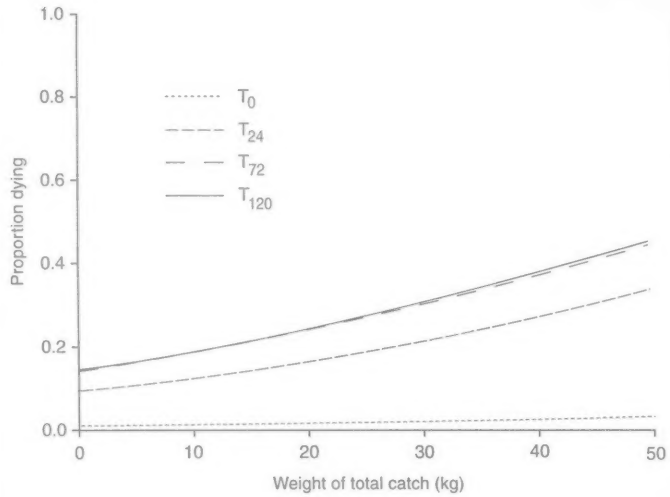


**Figure 4**

Logistic regression between catch weight and the expected proportion of school prawns (*Metapenaeus macleayi*) dying after being trawled in the Hunter River, sorted in the water tray, discarded into cages and sampled at four different times (immediately [$T_0$], and after 24 hours [$T_{24}$], 72 hours [$T_{72}$], and 120 hours [$T_{120}$]) in 2008.

When they initially contact the anterior sections of a trawl, prawns and other crustaceans with extended abdomens (e.g., Norway lobster *Nephrops norvegicus*) typically respond by contracting their tail muscle ventrally, effectively propelling themselves backwards into the top netting panels (Watson, 1976; Newland and Chapman, 1989). This behavior may be repeated several times, until an individual becomes fatigued, after which they often attempt to orientate towards the seabed using their swimmerets (Watson, 1989). Such activity is ineffective against the speed of the trawl, and therefore prawns are quickly forced against the meshes into the posterior trawl body and directed into the codend where they accumulate along with the rest of the catch.

The physiological responses supporting the above activity include anaerobic respiration and the rapid depletion of arginine phosphate in the tail muscle (Onnen and Zebe, 1983; Paterson et al., 1995). An end product of this exertion is lactic acid, which is removed during aerobic metabolism (Head and Balwin, 1986). In the present study, the concentrations of $_L$-lactate in live school prawns immediately sampled (i.e., at $T_0$) after being discarded from the various treatments in the Clarence River were elevated to mean levels between 14.2 ±2.0 and 15.6 ±1.5 μmol/g, that were greater than those previously observed for this species after exercise (typically <12 μmol/g), indicating that these individuals had been heavily exercising. Although some of the observed lactate accumulation could be attributed to onboard handling and associated anoxia (Hill et al., 1991), especially during the conventional procedures, the similar rate of accumulation across all treatments supports a strong, uniform, negative impact of the trawling process.

For some individuals, the physiological damage described above may have been sufficient to result in their immediate death, while for others the cumulative impacts of significant covariates, such as the weight of catch and salinity, would have contributed toward their more protracted (over 24 hours) trawl-related mortalities. In particular, as the catch accumulated, at least some school prawns probably sustained fatal injuries, including wounding and blood loss, due to pressure and abrasion. Also, the ongoing stimuli associated with repetitive contact among conspecifics and other organisms may have triggered additional bursts of anaerobic exercise, and further compromised their physiological condition. These density-dependant effects were more obvious in the Hunter than in the Clarence River, probably reflecting the considerably greater mean size of catch (19.4 kg [Hunter River]; 11.8 kg [Clarence River]), but also perhaps the slightly smaller mean size of school prawns (Table 1) and the presence of some soft individuals (i.e., 13% at $T_0$). Both of these latter factors have previously been demonstrated to increase the vulnerability of organisms to the impacts associated with discarding (Broadhurst et al., 2006).

Salinity also appeared to contribute towards mortality during trawling in the Clarence River. As with

other penaeids (e.g., western king prawns [*Penaeus latisulcatus*] [Sang and Fotedar, 2004] and fleshy prawns [*P. chinensis*] [Chen et al., 1995]), Maguire and Allan (1985) observed that although juvenile school prawns tolerate a range of salinities (from 1 to 30 psu), the mortality of captive individuals (in grow-out ponds) was negatively associated with salinities between 10 and 30 psu. On average, the salinities observed in the Clarence River were much lower than those of the Hunter River (5.4 vs. 10.2 psu) and possibly below the optimal range for osmoregulation. Although not necessarily fatal in the absence of other stressors, low salinities would require school prawns to maintain a comparatively greater metabolic rate to achieve osmotic balance, thereby increasing their susceptibility to other trawl-related impacts. Further, an abrupt reduction to even lower salinities during the retrieval of the trawl to the surface (as a consequence of the observed halocline) would require some readjustment of osmotic concentration to regulate tissue water. A similar transition through haloclines has been identified as a factor contributing towards mortality in other species, including Norway lobster (Harris and Ulmestrand, 2004), and probably had a cumulative impact on the stress, and ultimately mortality, of some school prawns.

It is difficult to accurately quantify the cumulative impact of all trawl-related stressors on total mortality, especially since the death of some control school prawns indicates the potential for natural mortality (which was assumed to be constant across all treatments). However, after monitoring the fate of individuals collected immediately after the codend was emptied (individuals that had minimal air exposure before being sorted), Macbeth et al. (2006) estimated a short-term (over three days) mortality of 16% directly attributable to 60-min trawls, which was only slightly greater than that determined for individuals escaping through codend meshes during trawling (11%, Broadhurst et al., 2002). The results from the present study support these estimates, with an adjusted, protracted mortality in the 2-min water tray (arguably the mildest treatment) of less than 21% for both rivers.

Assuming comparable trawl-induced mortalities across treatments, the remaining differences in fatalities can be attributed to the use of the water tray for minimizing some of the negative impacts associated with onboard handling, and especially with air exposure. Most likely, by facilitating aerobic respiration, the water tray would have allowed some school prawns to recover and restore argine phosphate levels and acid-base regulation (Taylor and Spicer, 1988), which probably helped to limit further physiological damage and mortality.

In addition to limiting air exposure, the water tray maintained temperature homeostasis. Although there was no significant effect of air temperature in the models applied to the conventional-tray treatments in either experiment, there was a significant negative relationship between cloud cover and mortality in the Clarence River ($P<0.05$). This association probably reflects the greater convection, heating, and subsequent desiccation of school prawns in direct sunlight on the dry tray. Gamito and Cabral (2003) observed similar effects of heating on the mortality of brown shrimp (*Crangon crangon*) and suggested that this could be reduced by using light-colored sorting containers and avoiding trawling during the hottest time of the day. Sorting in the water tray is probably a more effective alternative in New South Wales, although the lack of any impacts of cloud cover in the Hunter River, combined with the similar range of temperatures between the two rivers also illustrates the utility of shading the conventional sorting tray with an appropriate cover.

The only obvious limiting factor of the water tray was its size for the trawler working in the Hunter River. Vessels in the Hunter River typically catch fewer school prawns than those in the Clarence River, and therefore a smaller water tray was used in the Hunter River . It is possible that, in addition to the trawling-related impacts discussed above, large catches in the sorting tray on the Hunter River trawler contributed towards mortality as a consequence of their greater biological load. Although there was no significant impact of dissolved oxygen on mortality in the water tray, the mean dissolved oxygen recorded during the Clarence River deployments was higher than that in the Hunter (5.8 mg/L vs. 3.8 mg/L). Further, during one catch of 47 kg in the Hunter River, the dissolved oxygen in the water tray after 15 minutes of sorting was 2.4 mg/L. The potential for any negative effects associated with low dissolved oxygen could be simply reduced by increasing the water exchange or volume of the water tray, or both.

Although the results from this study indicate a significant reduction in discard mortality associated with using the water tray, it is important to remember that the discarded school prawns were protected in cages and therefore other collateral mortalities were not quantified. The levels of $_{L}$-lactate at $T_0$ indicated that alive, discarded school prawns were fatigued and, during conventional fishing, these individuals could be more susceptible to predation during their descent to the bottom (Lancaster and Frid, 2002). Further, because of the temporal increase in mortality, some school prawns may have maintained their vulnerability, particularly during the first 24 hours after having been discarded. The potential for these effects could be mitigated by subtle modifications to the water tray. In particular, because low (e.g., <15°C) water temperatures have been demonstrated to reduce metabolic activity in penaeids (Paterson, 1993), cooling the water tray may reduce some of the ancillary stressors identified above and help school prawns to recover more quickly. The concomitant use of a covered guiding panel that directs the discarded school prawns into the water behind the trawler could reduce predation.

Notwithstanding the need for some refinements, it is clear that using the water tray would eliminate most of the short-term mortalities associated with onboard handling, which would translate to a total reduction

in discard mortality of school prawns of more than a third. When combined with other selective mechanisms, such as square-mesh codends that reduce the catches of school prawns of nontarget sizes, the water tray should contribute towards the sustainability of trawling in New South Wales rivers.

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# The repulsive and feeding-deterrent effects of electropositive metals on juvenile sandbar sharks (*Carcharhinus plumbeus*)

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**Abstract**—Reducing shark bycatch and depredation (i.e., damage caused by sharks to gear, bait, and desired fish species) in pelagic longline fisheries targeting tunas and swordfish is a priority. Electropositive metals (i.e., a mixture of the lanthanide elements lanthanum, cerium, neodymium, and praseodymium) have been shown to deter spiny dogfish (*Squalus acanthias*, primarily a coastal species) from attacking bait, presumably because of interactions with the electroreceptive system of this shark. We undertook to determine the possible effectiveness of electropositive metals for reducing the interactions of pelagic sharks with longline gear, using sandbar sharks (*Carcharhinus plumbeus*, family Carcharhinidae) as a model species. The presence of electropositive metal deterred feeding in groups of juvenile sandbar sharks and altered the swimming patterns of individuals in the absence of food motivation (these individuals generally avoided approaching electropositive metal closer than ~100 cm). The former effect was relatively short-lived however; primarily (we assume) because competition with other individuals increased feeding motivation. In field trials with bottom longline gear, electropositive metal placed within ~10 cm of the hooks reduced the catch of sandbar sharks by approximately two thirds, compared to the catch on hooks in the proximity of plastic pieces of similar dimensions. Electropositive metals therefore appear to have the potential to reduce shark interactions in pelagic longline fisheries, although the optimal mass, shape, composition, and distance to baited hooks remain to be determined.

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The worldwide bycatch of sharks is estimated to be 260,000–300,000 metric tons annually (11.6 to 12.7 million individual sharks) (Bonfil, 1994; Camhi et al., 1998). In pelagic longline fisheries targeting tunas and swordfish, it is not uncommon for the number of sharks caught to exceed that of the desired fish species (Stevens, 1992; Bonfil, 1994; Gilman et al., 2008). Shark populations are especially vulnerable to high rates of fishing mortality because of their slow growth rates, low reproductive output, and late sexual maturity. Once depleted, they also generally have slow rates of recovery because of these characteristics (Smith and Snow, 1998; Chen and Yuan, 2006). Scalloped hammerhead (*Sphyrna lewini*), oceanic whitetip (*Carcharhinus longimanus*), and tiger shark (*Galeocerdo cuvier*) populations have already decreased within the range from 60% to 99% of their historical biomass (Baum et al., 2003; Baum and Myers, 2004; Gilman et al., 2008), and these species are now included on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN, 2008). Such severe reductions in elasmobranch populations have the potential to detrimentally restructure marine ecosystems (Jackson et al., 2001; Myers and Worm, 2003; Worm et al., 2006; Myers et al., 2007). Survival rates of pelagic sharks released from longline gear appear high for animals that are not moribund when the gear is retrieved (Moyes et al., 2006). Nonetheless, reduction of both shark bycatch and depredation (i.e., shark damage to longline gear, bait, and desired fish species) is considered a priority (Gilman et al., 2008, Mandelman et al., 2008).

Sharks (but not the large pelagic teleosts targeted by longline fisheries) possess a unique sensory system based on the ampullae of Lorenzini that can detect electric field gradi-

ents as small as 5 nV/cm (Haine et al., 2001). These ampullary receptors are most sensitive to frequencies from 1 to 8 Hz (Montgomery, 1988), are capable of detecting weak electric fields generated by neuromuscular activity, and can guide sharks to prey in the absence of other sensory stimuli (Kajiura and Holland, 2002; Kajiura, 2003; Collin and Whitehead, 2004). It should be possible, therefore, to develop effective deterrent procedures that could take advantage of the sharks' electroreceptive sense. The procedures could then decrease the bycatch and incidental mortality of sharks and increase fishing efficiency and yield of the desired fish species. Strong electric fields have been shown to deter approaching sharks, presumably by overloading their electrosensory modality (Smith, 1974, 1991; Cliff and Dudley, 1992). However, currently available electronic devices for achieving this behavioral response are designed to protect humans and aquaculture structures from shark attack and are large, expensive, and not practical for deployment on longline fishing gear. There are no data on the minimum field strength needed to achieve electrosensory repulsion.

Electropositive metals (generally mixtures of the lanthanide elements praseodymium, neodymium, cerium, lanthanum, samarium, and yttrium) rouse juvenile lemon sharks (*Negaprion brevirostris*), nurse sharks (*Ginglymostoma cirratum*), and spiny dogfish sharks (*Squalus acanthias*) from tonic immobility when brought close to the head (Stoner and Kaimmer, 2008). Electropositive metals have also been shown to deter spiny dogfish sharks from attacking baits in a tank study (Stoner and Kaimmer, 2008), and to reduce the catch of this species by 19% on bottom longline gear (Kaimmer and Stoner, 2008). Electropositive metals are assumed to stimulate the electroreceptive system by giving up cations to the more electronegative skin of the elasmobranchs (Rice, 2008; Stoner and Kaimmer, 2008), although the exact mechanisms responsible for repulsion are not known.

Our studies are designed to determine if electropositive metals affect the behaviors of juvenile sandbar sharks (*Carcharhinus plumbeus*) under both laboratory and field conditions. Sandbar sharks are highly suitable for this line of research because they do well and feed readily in captivity. They are also an obligatory ram-ventilating species and their constant forward motion makes it easier to measure changes in swimming patterns caused by electropositive metals, compared to species that remain motionless on the bottom for extended periods. More importantly, although primarily a coastal species (Conrath, 2005; Conrath and Musick, 2008), the sandbar shark is a member of the family *Carcharhinidae* (requiem sharks), which includes many of the other shark species that frequently interact with pelagic longline gear (Mandelman et al., 2008). Results with sandbar sharks should, therefore, provide a good indication of the efficacy of electropositive metals for reducing shark bycatch in pelagic longline fisheries.

Our experiments with captive sandbar sharks include tests of the ability of electropositive metals to influence the swimming patterns of individual animals in the absence of food motivation and to repel sharks from pieces of cut bait. The former is intended to quantify repulsive distances, and both are intended to provide data directly comparable with those obtained previously with spiny dogfish sharks (Stoner and Kaimmer, 2008; Tallack and Mandelman, in press). Our deployment of longline fishing gear in a tidal lagoon system used as a nursery area by juvenile sandbar sharks (Conrath, 2005; Conrath and Musick, 2007) tested the ability of electropositive metal to deter sharks under field conditions and provided data comparable to data from recent studies where spiny dogfish sharks were targeted by a similar method (Kaimmer and Stoner, 2008; Tallack and Mandelman, in press).

## Materials and methods

Experiments with captive animals were conducted during the summer months (June through August 2007) at the Virginia Institute of Marine Science, Eastern Shore Laboratory, in Wachapreague, Virginia. Juvenile sandbar sharks weighting up to ~5 kg (i.e., neonates to approximately 5 years old; Casey and Natanson, 1992) were captured with standard recreational hook-and-line fishing gear in the surrounding tidal lagoon system and transported to an outdoor circular fiberglass tank (7 m diameter, 1.8 m deep) as described previously (Brill et. al., 2008). The tank was supplied with sea water pumped from the adjacent tidal lagoon which was passed through sand filters to remove suspended particles, as well as phytoplankton and fouling organisms. Water from the holding tank was also continuously circulated through a separate set of sand filters, ultraviolet sterilizer, biofilter, and protein skimmer. Tank temperature and salinity over the course of the study (22–29°C and 30–33‰, respectively) reflected that of the adjacent tidal lagoon. When not part of an active experiment, the sharks were fed pieces of cut menhaden (*Brevoortia tyrannus*) every other day. All sharks were actively feeding before use in any trials.

### Repulsion experiments with individual sharks

Experiments were performed on 10 sharks, and individuals were not used more than once. For each replicate, an individual shark was transferred from the main holding tank to a smaller vinyl circular indoor test tank (3.6 m diameter, 0.67 m water depth) and allowed to acclimate for 24 hours. The test tank was supplied with seawater pumped from the adjacent tidal lagoon which was passed through sand filters. Temperature and salinity ranged from 22° to 29°C and from 30‰ to 33‰ over the course of the study.

An experiment consisted of three one-hr periods. At the start of the first hour, a string of three lead fishing weights was suspended in the tank to allow the shark

to acclimate to the presence of a new visual stimulus. At the start of the second hour the string of lead fishing weights was quietly removed and immediately replaced with either a string of three electropositive metal bars, or the string of lead fishing weights was placed back into the tank. This choice was randomized. At the start of the third hour, the string of electropositive metal bars or lead fishing weights was removed and replaced with the other. Only the video records from the second and third hours (i.e., one hour in the presence of electropositive metal bars and one hour in the presence of lead fishing weights) were subsequently analyzed.

The three electropositive metal bars (~2 cm × 2 cm × 10 cm) comprised neodymium (76%), praseodymium (23%), and minor amounts (<0.04%) of cerium, lanthanum, samarium, and yttrium (Hefa Rare Earth, Vancouver, Canada). The three lead fishing weights had similar dimensions to those of the electropositive metal bars. The strings electropositive metal bars and lead fishing weights were constructed by using single pieces of nylon monofilament fishing line and were suspended in the tank at a position approximately 35 cm from the tank sidewall (Fig. 1). This lateral position was chosen because preliminary observations had shown that juvenile sandbar sharks swam predominately in a circular pattern near the tank wall. There was sufficient space, however, for the fish to pass easily between the nylon line (holding the electropositive metal bars or lead fishing weights) and the tank wall. Individual electropositive metal bars and lead fishing weights were attached to the nylon fishing line so as to be at approximately 16, 32, and 48 cm below the surface when suspended in the tank.

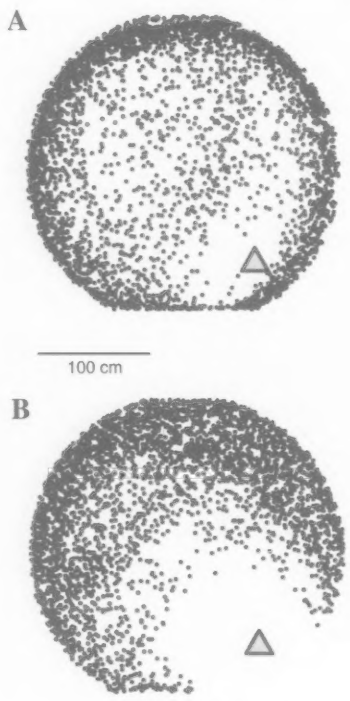


**Figure 1**

Positions of a juvenile sandbar shark (*Carcharhinus plumbeus*) at 1-sec intervals obtained with Lolitrack automated video analysis software (Loligo Systems, Tjele, Denmark). Three lead fishing weights (**A**) or three electropositive metal bars (**B**) were suspended in the tank using monofilament fishing line at the position indicated by the triangles. The video record was acquired with a digital video camera mounted directly above the center of a vinyl circular tank (3.6 m diameter, 0.67 m water depth). Small portions of the tank at the 12 o'clock and 6 o'clock positions were out of frame because of the maximum available height of the laboratory ceiling where the video camera was positioned.

A digital monochrome video camera (IDS Imaging Development Systems Inc., Cambridge, MA) equipped with a wide angle lens was used to acquire a continuous record (on a laptop computer) of the swimming patterns of each shark. The camera was mounted on the laboratory ceiling, over the center of the tank, approximately 1.5 m above the water surface. This allowed an almost complete view of the tank, although small areas at the 12 and 6 o'clock positions remained out of frame because of the maximum height of the digital video camera imposed by the laboratory ceiling. The locations of the sharks were subsequently digitized ($x$, $y$ coordinate system) at one-second intervals from the video record by using Lolitrack automated video analysis software (Loligo Systems, Tjele, Denmark). The software generally digitized the broadest area of the shark from the dorsal view (i.e., the area between the pectoral fins and first dorsal fin).

Shark positions were translated into quantifiable behaviors by calculating the distances between the sharks and the electropositive metal or lead weights from the one-second interval location records. These data were summarized by compiling frequency distributions with 5-cm bins. Fractional values for each distance bin were calculated from the total number of position estimates for each animal when the electropositive metal bars or lead fishing weights were present in the tank. The fractional bins were averaged across all fish. A two-way (treatment×distance bin) repeated measures analysis of variance (ANOVA) procedure was used to test for differences in the frequency distributions (with the use of arcsine transformed percentage data), with *post hoc* tests for significant differences between individual bins (Sigma Stat, vera. 3.0.1, Systat Software, Inc., San Jose, CA). The significance level for all tests was $P < 0.05$.

The digital position records were also used to calculate swimming speeds, which were subsequently segregated into swimming speeds recorded when the fish was within 100 cm of the electropositive metal bars or lead fishing weights, and into swimming speeds recorded

when the fish was further than 100 cm from the electropositive metal bars or lead fishing weights.

### Feeding deterrent experiments

Groups of sharks maintained in the outdoor circular fiberglass holding tank were used to determine the ability of electropositive metals to deter sharks from attacking bait. Individual pieces of cut menhaden were placed 30 cm below a single electropositive metal bar (~2 cm × 2 cm × 10 cm and of the same composition described previously) by using a monofilament nylon fishing line. For control trials, pieces of cut menhaden were placed 30 cm below a stainless steel bolt of approximately the same dimensions as the electropositive metal bar. Baits were attached to the monofilament line with light twine that allowed the bait to be removed by the sharks with moderate effort. Hooks were not used because of the risk of injuring the sharks and the likelihood that hooking would influence the willingness of the sharks to attack baits in subsequent trials. The line (with the bait and stainless steel bolt or electropositive metal bar) was suspended near the center of the tank and in approximately the middle of the water column. The order of presentation was randomized.

During each trial, the line was immediately removed from the water after the bait was attacked and the time from presentation to attack was recorded. The line was also removed from the water if the bait was not attacked within three minutes. In either case, five minutes were allowed before the next trial was begun. Because of the number of sharks in the tank, the rapidity of the attacks, and the frequent shark-shark interactions, it was not possible to identify which individual attacked the bait or to quantify specific changes in behavior as the bait was approached.

Two separate series of experiments were conducted. In the first, 14 actively feeding juvenile sandbar sharks were present in the holding tank and 14 trials (seven with the electropositive metal and seven with the stainless steel bolt) were conducted every other day over a 14-d period. The sharks were fed to satiation at the end of each set of trials, but not on the days between experiments. Two additional sessions were run one week after the completion of the first 14 sessions. The original group of sharks was then released and replaced with seven naive individuals. Eight trials (four with the electropositive metal bar and four with the stainless steel bolt) were conducted every other day, over a 12-day period. As in the previous experiments, the sharks were fed to satiation at the end of each set of trials, but not on the days between experiments. Feeding trials were run at approximately the same time everyday (late afternoon).

### Longline experiments

Bottom longline fishing gear was used to test the ability of electropositive metal to influence shark catch rates in the field. Longline trials were conducted during the summer months (July and August 2008). The gear was deployed a total of 26 times (two deployments per day) and all deployments except for one were in the tidal lagoon system adjacent to the eastern shore of Virginia (an area of tidal creeks and broad marshes separated from the Atlantic Ocean by a series of barrier islands to the east). One longline set was made in the ocean immediately offshore of the barrier islands. All longline sets were conducted during daylight hours and the gear retrieved after two hours when conditions permitted. Gear deployment schedules were primarily based on weather, as well as crew and vessel availability, rather than on time of day or tidal state. Experiments were generally not undertaken on consecutive days.

Approximately 40 hooks were deployed during each set. The monofilament dropper lines were two meters long and were terminated with steel circle hooks (10/0 or 11/0) baited with pieces of menhaden. Dropper lines were connected to the mainline at 10-m intervals to help ensure that each dropper fished independently. Small pieces (approximately 30–70 g initial weight) of electropositive metal comprising lanthanum (28%), cerium (53%), neodymium (15%), and praseodymium (4%) were attached to the dropper lines approximately 10 cm from the hook by using plastic zip ties. The electropositive metal pieces, cut in cross section from the ingots supplied by the distributor (Hefa Rare Earth, Vancouver, Canada), were approximately 2-cm thick plates (~30–60 $cm^2$ surface area per side). Plastic pieces, of approximately the same shape and surface area were attached at the same positions to control for any visual deterrent or mechanical effects. Lines with electropositive metal near the hook and with a plastic piece near the hook were attached to the mainline in an alternating pattern and in equal numbers during each gear deployment. This arrangement allowed the resultant catch data to be analyzed with a chi-square procedure based on the expectation that equal numbers of sharks would be caught on hooks near a plastic piece or on hooks near electropositive metal, if the latter did not alter shark behaviors.

Captured sharks were brought into the boat, hooks were cut in two places to help ensure that they would be shed quickly, standard length was measured, and sex was noted. These sharks were then immediately released. Clearnose skates (*Raja eglanteria*) were treated similarly. Large rays (orders: Rajiformes and Myliobatiformes) were released without removing them from the water to ensure crew safety, and were therefore usually not identified to species.

## Results

### Repulsion experiments with individual sharks

In the presence of the lead fishing weights, sharks swam predominately around the periphery of the tank, showed essentially no avoidance response, and fre-

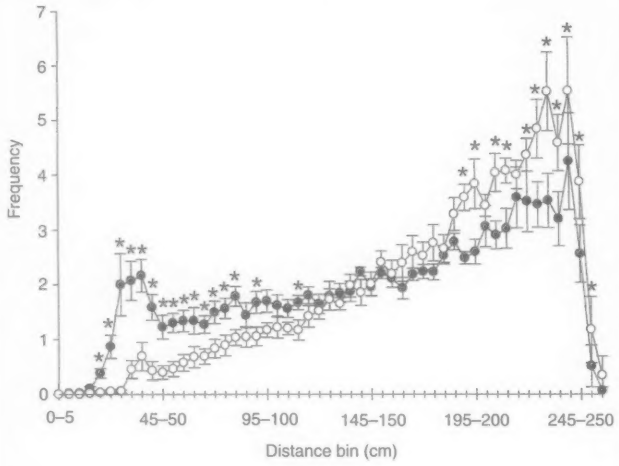


**Figure 2**

Frequency distributions (based on 5-cm bins) of the observed distances in relation to lead fishing weights (filled circles) or electropositive metal (open circles) calculated at 1-sec intervals from the video records of juvenile sandbar sharks (*Carcharhinus plumbeus*, $n$=10). When compared to the lead weights, significantly fewer (indicated by *, two-way repeated measures ANOVA, $P$ <0.05) shark positions were recorded within 100 cm of electropositive meta, whereas more positions were recorded at distances more than approximately 200 cm from electropositive metal.

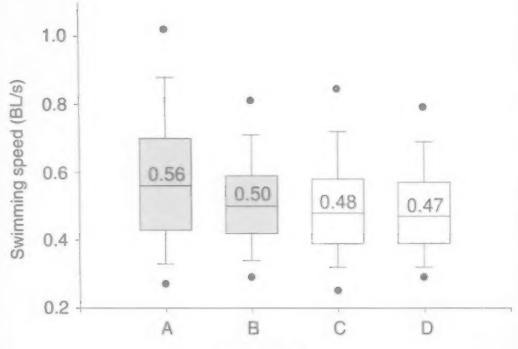


**Figure 3**

Median swimming speeds in body lengths per second (BL/s) when juvenile sandbar sharks (*Carcharhinus plumbeus*) were within 100 cm of electropositive metal (**A**), more than 100 cm from electropositive metal (**B**), within 100 cm of lead fishing weights (**C**), and more than 100 cm from lead fishing weights (**D**). The high variability precluded statistically significant differences, but there appears to be a trend for fish to swim faster in proximity to electropositive metal than under the other circumstances. Limits of boxes show the 25$^{th}$ and 75$^{th}$ percentiles, the error bars the 90$^{th}$ and 10$^{th}$ percentiles, and the single points the 95$^{th}$ and 5$^{th}$ percentiles.

quently passed between the lead fishing weights and tank wall. In contrast, sharks generally avoided approaching electropositive metal bars, which precluded them from passing between the electropositive metal bars and the tank wall. The locations of a single sandbar shark typifying these behaviors are shown in Figure 1.

The frequency distributions of positions in relation to the lead fishing weights or electropositive metal bars demonstrate avoidance of the latter by the sharks (Fig. 2). Significantly fewer positions were recorded within approximately 100 cm of the electropositive metal bars, and significantly more positions at the maximum distances (further than approximately 200 cm from the electropositive metal bars). The frequency distributions under both circumstances increased with distance and truncated sharply at the greatest distances because of simple positional geometry and the limitations imposed by the dimensions of the circular test tank.

The swimming speed data were not normally distributed and are therefore shown as box and whisker plots (Fig. 3). There appeared to be a tendency for swimming speeds to be greater in proximity to the electropositive metal bars than under the other circumstances. However, the small differences and extreme variability precluded statistically significant differences.

### Feeding deterrent experiments

Bait pieces located approximately 30 cm from a stainless steel bolt were generally attacked within 30 seconds of presentation (Figs. 4 and 5). In contrast, sharks did not attack baits located in proximity of an electropositive metal bar within three minutes, at least during the initial trials. When the repulsive effect was evident, sharks would rapidly approach the bait, flinch, turn sharply, and rapidly depart. Although we were not able to quantify these behaviors, they matched the responses of spiny dogfish sharks under similar circumstances described by Stoner and Kaimmer (2008) and mirrored the apparent changes in swimming speeds of individual sharks near electropositive metal (Fig. 3).

When 14 sharks were present in the tank (Fig. 4), the repulsive effect extinguished fairly suddenly during day 2, but reappeared during the initial trials on day 4, and again during the initial trials on days 10 and 21. Because it was impossible to identify individuals, it is unknown if only one or a few sharks overcame the deterrent effect of electropositive metal. The repulsive effect did not reappear after a one-week period where trials were not run; indicating that once tolerance of electropositive metal is learned it is retained at least over the short term.

During the second set of experiments with fewer sharks in the tank (seven animals versus 14), the deterrent effect of electropositive metal was apparent until day 8 and it did not completely disappear until day 12 (Fig. 5).

### Longline trial experiments

Of the juvenile sandbar sharks captured, 39 were female, 26 were male, and there was one individual where sex was not recorded. Sharks ranged in size (standard length) from 47 to 130 cm, and had a median length of 72.5 cm. Only one cownose ray (*Rhinoptera bonasus*) was captured, the other rays were either *Gymnura* spp. or *Dasyatis* spp. Two Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) and nine clearnose skates (*Raja eglanteria*) were captured during the one gear deployment made outside the lagoon system.

The ratio of sharks caught on hooks near plastic to sharks caught on hooks near electropositive metal (2.6:1) was significantly different from the predicted ratio of 1:1 if the presence of the latter had no deterrent effect (chi square test $P$=0.001, df=1, $\chi^2$=10.78). In other words, electropositive metal near the hooks reduced the catch rates of sharks by 62% (Table 1). In contrast, the numbers of rays caught on hooks near plastic and on hooks near electropositive metal were not significantly different from the expected ratio of 1:1 (chi square test $P$=0.67, df=1, $\chi^2$=0.39), indicating that the presence of electropositive metal had no deterrent effect. The low number of clearnose skates captured precluded any definitive conclusions. However, the essentially equal numbers of skates caught on each hook type (Table 1) implies that the presence of electropositive metal does not deter this species.

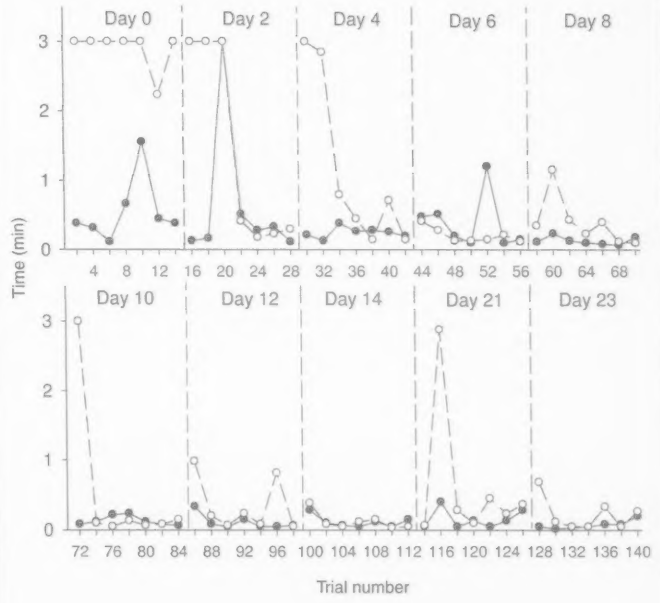


**Figure 4**

Time taken for cut pieces of menhaden (*Brevoortia tyrannus*) to be attacked when suspended 30 cm below a stainless steel bolt (filled circles) or an electropositive metal bar (open circles). The line was removed from the tank if the bait was not attacked within three minutes. Trials were conducted with 14 juvenile sandbar sharks (*Carcharhinus plumbeus*) maintained in a circular fiberglass tank (7 m diameter, 1.8 m deep). Fourteen trials (seven with the electropositive metal bar and seven with the stainless steel bolt) were conducted every other day for the first 14 days, suspended for seven days, and then two additional trials were run to test whether the electropositive metal near the bait, as seen during the initial trails, would continue to deter the sharks.

## Discussion

### Repulsion experiments with individual sharks

Because juvenile sandbar sharks showed no reactions to lead fishing weights (other than to avoid running into them), we concluded that lead fishing weights exert no significant repulsive effect. In contrast, juvenile sandbar sharks generally avoided approaching the electropositive metal bars presumably because they produce mild irritation. Whether this irritation is chemical or electrical (i.e., stimulation of the sharks electroreceptive system) is unknown. Given the apparent definitive

**Table 1**

Catch of sharks (primarily juvenile sandbar sharks, *Carcharhinus plumbeus*), rays (primarily *Gymnura* spp. and *Dasyatis* spp.) and clearnose skates (*Raja eglanteria*) by bottom longline gear. Pieces of electropositive metal, or pieces of plastic of similar dimensions, were placed within 10 cm of the hooks. Hooks in proximity to electropositive metal or to plastic pieces were deployed in equal numbers and in an alternating pattern during each set.

| | Sharks | Rays | Skates |
|---|---|---|---|
| Hooks near electropositive metal | 16 | 10 | 4 |
| Hooks near plastic | 42 | 13 | 5 |

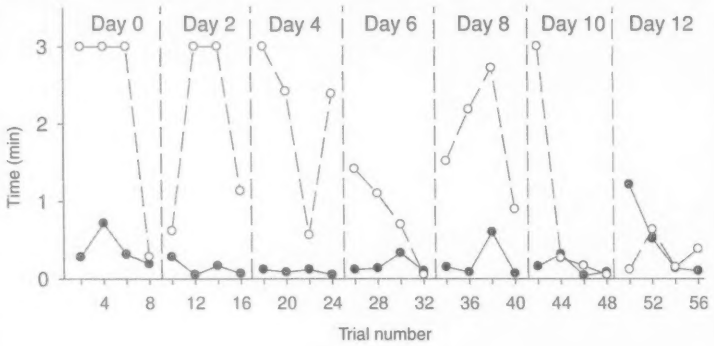


**Figure 5**

Time taken for cut pieces of menhaden to be attacked when suspended 30 cm below a stainless steel bolt (filled circles) or an electropositive metal bar (open circles). The line was removed from the tank if the bait was not attacked within three minutes. During these trials, seven juvenile sandbar sharks (*Carcharhinus plumbeus*) were maintained in a circular fiberglass tank (7 m diameter, 1.8 m deep). Eight trials (four with the electropositive metal bar and four with the stainless steel bolt) were conducted every other day. The deterrent effect of electropositive metal was present over a greater number of days compared with the previous situation when 14 sharks were present in the tank. The difference is assumed to be due to increased competition for food which limited the repulsive effect of electropositive metal in the latter circumstance.

boundary of the area that is avoided (Fig. 1), we surmise the latter to be the case. Stoner and Kaimmer (2008) reach similar conclusions with respect the deterrent effect of electropositive metal on spiny dogfish sharks. The effective range of deterrence (~100 cm) for juvenile sandbar sharks is, however, considerably larger than that for spiny dogfish sharks (10–20 cm) (Stoner and Kaimmer, 2008). Whether this is due to differences in water temperatures (~10°C for spiny dogfish sharks versus 22–29°C for juvenile sandbar sharks), mass or shape of bars, specific composition of the electropositive metals, or species differences remains to be determined. The number of electrosensory pores present in sandbar sharks is approximately twice that in spiny dogfish sharks (2317 versus 1262, respectively; Cornett, 2006) which may explain the difference in the distances that these sharks were deterred.

Because of the limited range of deterrence, electropositive metal would have to be placed near every hook in pelagic longline gear, although it appears that it could be placed at distances that are unlikely to interfere with capture of the targeted fishes. It is unknown if electropositive metal could protect hooked fishes from depredation by sharks, which is a significant problem (Gilman et al., 2008; Mandelman et al., 2008).

### Feeding deterrent experiments

Stoner and Kaimmer (2008) theorize that the presence of electropositive metal is irritating or possibly interferes with the ability of sharks to locate a food item. We hypothesize that irritation is the more likely reason the bait was not attacked within three minutes during the initial trials with electropositive metal in our feeding experiments. The tank was brightly lit and the water was essentially free of suspended particles because of the extensive filtration. We therefore contend that the sandbar sharks located the bait primarily by vision (although olfaction may also be involved). Moreover, the pieces of cut menhaden would obviously not have the bio-electric signals emitted by living organisms (Haine et al., 2001). Further investigation into the exact mechanism(s) underlying the effect of electropositive metals as is clearly warranted.

Our specific experimental procedures were designed to ensure that feeding motivation remained high and thus to minimize the influence of feeding motivation on our results. Any influence of competition on feeding motivation could not be controlled however, except by altering the number of sharks in the tank. Competition is well known to increase feeding motivation (Ryer and Olla, 1991; Eklov, 1992) and we assume that it likewise lessens the deterrent effects of electropositive metal. Increased feeding motivation due to competition could, therefore, explain the short-lived deterrent effects of electropositive metal when 14 sharks are present in the tank. Our observation that the deterrent effect lasts longer during the trials when only seven sharks are present supports this contention.

Our data also imply that tolerance of electropositive metals can be learned, and that this learned behavior is retained for at least seven days. It is unknown how often individual sharks encounter pelagic longline gear, but it is unlikely to be anywhere near the frequency of our feeding trials with captive sandbar sharks. For this reason we propose that learned tolerance of electropositive metals will unlikely diminish their deterrent effect when used with pelagic longline fishing gear.

### Longline trial experiments

From our longline catch data (Table 1), it is clear that the presence of electropositive metal near hooks is a strong deterrent to juvenile sandbar sharks, but not to rays. In recent studies where similar methods were used resulted in either a smaller reduction in catch rates (20%) of spiny dogfish sharks (Kaimmer and Stoner, 2008) than we observed, or in no statistically significant reduction (Tallack and Mandelman, in press). Surprisingly, Kaimmer and Stoner (2008) also recorded a large reduction (46%) in the catch of longnose skates (*Raja rhina*) due the presence of electropositive metal near longline hooks, whereas we saw no indication of a repulsive effect on clearnose skates.

The sensitivity of the electroreceptor system has been studied in a broad range of elasmobranchs (reviewed by Montgomery, 1988; Kalmijn, 2003) and there is no evidence of a lesser sensitivity in rays when compared to sharks. More specifically, the sensitivity of the electroreceptor system in the sandbar shark, the blacktip reef shark (*Carcharhinus melanopterus*, family Carcharhinidae), and the mangrove whipray (*Himantura granulata*, family Dasyatidae) are roughly equivalent (1 to 4 nV/cm; Haine et al., 2001; Kajiura and Holland, 2002). By implication, therefore, the catch rates of all the elasmobranch species interacting with the longline gear should be reduced equally, but clearly are not. The species-specific responses of sharks, skates, and rays to electropositive metal may reside at the receptor level (Tricas and New, 1998), the level of central processing, or simply reflect different behavioral tolerance related to feeding motivation. Kaimmer and Stoner (2008) and Tallack and Mandelman (in press) both speculate that the abundance of dogfish results in strong competition for food and increased aggressiveness, and that these limit the repulsive effect of electropositive metal. Our results showing a longer lasting repulsive effect of electropositive metal during feeding experiments when fewer sharks are present in the tank (Fig. 4 and 5) support this contention. Assessing the specific differences between various species of sharks, skates, and rays could clearly be a fruitful area of investigation.

### Health and environmental safety concerns with use of electropositive metals in fisheries

The electropositive metals used in our experiments are mixtures of lanthanide elements (e.g., lanthanum, cerium, neodymium, and praseodymium) that are collectively known as the "rare earth" elements, although they are not particularly rare (Bulman, 1994). Lanthanide elements are generally considered nontoxic to mammals primarily because they are not easily absorbed if ingested (Haley, 1965; Bulman, 1994). Their accumulation in animal tissue is therefore generally very low to negligible even for animals in long-term feeding trials, and transfer to humans through foodstuffs is likewise very low (Redling, 2006). We therefore conclude that the use of electropositive metals as elasmobranch deterrents would pose little if any toxicity to fishing crews handling the material, or to the food safety of targeted fish species. Lanthanide elements are also used as crop fertilizers and animal feed performance boosters for poultry, sheep, cattle, pigs, fish, and prawns; and in a variety of medical applications such as antimicrobial agents, MRI imaging, burn and cancer treatments, and for countering hyperphosphatemia in renal dialysis patients (Fricker, 2006).

Lanthanide elements injected intravenously can be toxic, however, because they cross cell membranes by passing through calcium channels, and because they have high affinity for calcium binding sites on biological molecules (Haley, 1965; Bulman, 1994). It is therefore at least possible that extensive distribution of lanthanide elements in the marine environment could impact invertebrate species (e.g., mollusks and crustaceans) that routinely incorporate calcium into their shells and exoskeletons.

### Conclusion and future directions

Improving gear selectivity (i.e., reducing shark bycatch and depredation) is considered a high priority in pelagic longline fisheries because of its ecological and economic benefits (Gilman et al., 2008, Mandelman et al., 2008). The use of electropositive metals appears promising in this regard. However, the specific composition, mass, and shape of the composite metal deterrent representing an optimal compromise between a high deterrent effect and a long useable durability in seawater remain to be ascertained. In conjunction with at-sea trials, behavioral assays with captive juvenile sandbar sharks would provide an effective means for testing and optimizing the use of electropositive metals.

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of William and Mary Institutional Animal Care and Use Committee and comply with all current applicable laws of the United States of America. This is contribution 2991 from the Virginia Institute of Marine Science.

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# Effects of a large fishing closure on benthic communities in the western Gulf of Maine: recovery from the effects of gillnets and otter trawls

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**Abstract**—The recovery of benthic communities inside the western Gulf of Maine fishing closure area was evaluated by comparing invertebrate assemblages at sites inside and outside of the closure four to six years after the closure was established. The major restriction imposed by the closure was a year-round prohibition of bottom gillnets and otter trawls. A total of 163 seafloor sites (~half inside and half outside the closure) within a 515-km$^2$ study area were sampled with some combination of Shipek grab, Wildco box corer, or underwater video. Bottom types ranged from mud (silt and clay) to boulders, and the effects of the closure on univariate measures (total density, biomass, taxonomic richness) of benthos varied widely among sediment types. For sites with predominantly mud sediments, there were mixed effects on inside and outside infauna and no effect on epifauna. For sites with mainly sand sediments, there were higher density, biomass, and taxonomic richness for infauna inside the closure, but no significant effects on epifauna. For sites dominated by gravel (which included boulders in some areas), there were no effects on infauna but strong effects on epifaunal density and taxonomic richness. For fishing gear, the data indicated that infauna recovered in sand from the impacts of otter trawls operated inside the closure but that they did not recover in mud, and that epifauna recovered on gravel bottoms from the impact of gillnets used inside the closure. The magnitudes of impact and recovery, however, cannot be inferred directly from our data because of a confounding factor of different fishing intensities outside the closure for a direct comparison of preclosure and postclosure data. The overall negative impact of trawls is likely underestimated by our data, whereas the negative impact of gillnets is likely overestimated.

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The Western Gulf of Maine (WGOM) closure area was implemented by the National Marine Fisheries Service on 1 May 1998 as part of an overall effort to rebuild overfished New England groundfish stocks such as cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), other gadids, and flatfish. The WGOM closure is one of the largest year-round closures in the United States, designed to protect habitat and help in the recovery of overfished species. The major restriction imposed by the closure was year-round prohibition of commercial fishing gear that is capable of capturing groundfish, principally otter trawls and gillnets. A variety of other fishing gears (e.g., lobster pots, recreational hook-and-line gear), however, have been deployed in the area since its establishment. The WGOM closed area fits the definition of a marine protected area (MPA) because it provides some level of protection of habitat and resources (NRC, 2002). In many areas, MPAs have been useful management tools but the causal mechanisms for their effectiveness in protecting habitat and meeting other management objectives differ widely, largely because MPAs vary widely in design and other characteristics (Fogarty and Murawski, 2005; Shipley, 2004). Hence, there is a need to assess individual MPAs in the context of environmental and other characteristics, as well as in regard to the particular combination of restrictions placed on each area. For assessing the data presented here, the closure was considered as an area that was potentially recovering from the impacts of gillnets and otter trawls.

There have been no published studies on the effects of the WGOM closure on seafloor habitats, and none designed specifically to address the effects of the closure on groundfish

stocks. Recent analyses of commercial trawl data (Murawski et al., 2004, 2005) have shown no appreciable effect of the closure on stock rebuilding in the region, although there has been substantial recovery of some groundfish stocks since the closure was implemented. From studies of MPAs in other areas, for example, New England (e.g., Collie et al., 2005), it seems reasonable to expect that a closure of the magnitude of the WGOM area would have a measurable effect on habitat recovery and fish stock rebounds. Its role in these respects, however, remains to be demonstrated.

In this article, we present data from grab and box core samples of sediments and infauna, underwater video surveys of benthic fauna, and general seafloor conditions four to six years after the closure of WGOM, all of which indicate that the closure has resulted in substantial recovery of some bottom habitat types. We offer hypothetical causes for the recovery in the context of gear removal, and we discuss the implications for ecosystem-level management of the closure.

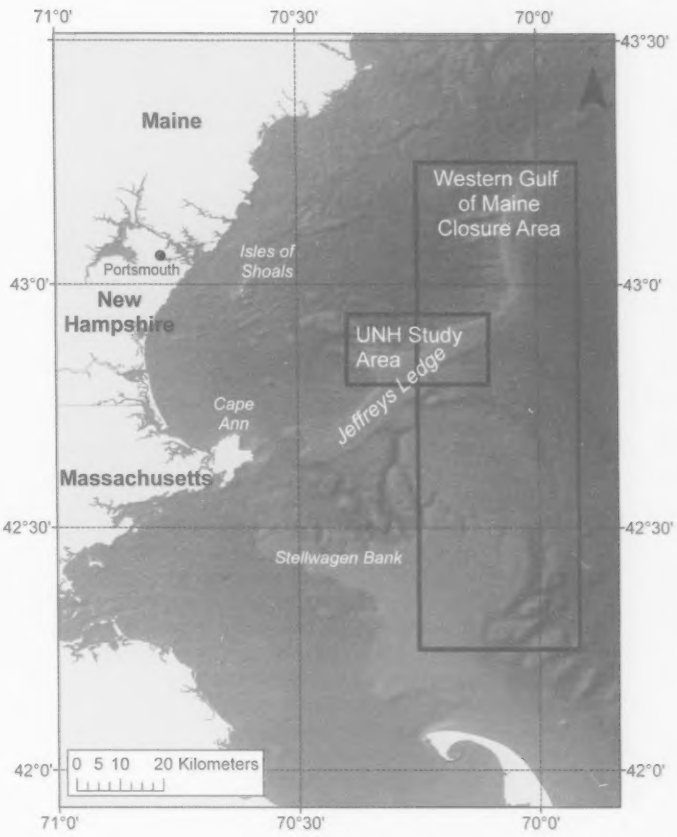


**Figure 1**

Location of the 515 km$^2$ University of New Hampshire study area (UNH Study Area) in relation to the western Gulf of Maine closure area. The map has a resolution of 90-m to one pixel and indicates bathymetric contours with light gray (shallow water) and dark gray (deep water).

## Materials and methods

### Study area

The study was restricted to a 515-km$^2$ area that comprised a section of the central western WGOM closed area and a section outside the closed area (Fig. 1). The overall closure area, which covers much of Jeffreys Ledge, is about 30 km wide (east–west) and 110 km long (north–south) and is located off the southern Maine, New Hampshire, and northern Massachusetts coasts. The location of the study area was chosen mainly because it has similar environmental characteristics both inside the closure and outside the closure.

A 5-m pixel resolution bathymetric map covering about 85% of the area and produced from multibeam sonar data collected between December 2002 and January 2003 (Malik and Mayer, 2007) was used as a general base map for the present study. It functioned as a guide for the final selection of sampling-site locations and for the interpretation of data related to potential gear impacts on bottom habitats.

An important feature of the study area for our research was the level of fishing activity, historically as well as after implementation of the closure. Data on date, location, and gear type (acquired by the National Marine Fisheries Service from federally permitted commercial fishing vessels) were acquired and plotted. For map production, the raw data (which were submitted at 1-min intervals of longitude and latitude) were plotted. All locations with fewer than five trip records were deleted from our analysis to eliminate potentially spurious data arising from reporting mistakes or for other reasons. Vessel trip report data from 4.5-yr preclosure (1994–98) and postclosure (1998–2002) were obtained for all reported gear types. Bottom gillnets and otter trawls were represented in >95% of the data records, and therefore other gear types were not analyzed.

### Study design

The study was a control-impact assessment (Osenberg et al., 1994) for which there was systematic sampling of the seafloor at approximately equal numbers of sites distributed inside and outside the closed area (Fig. 2). Sampling sites were located on a grid with sampling points at approximately 1.3-km intervals (0.75 min of

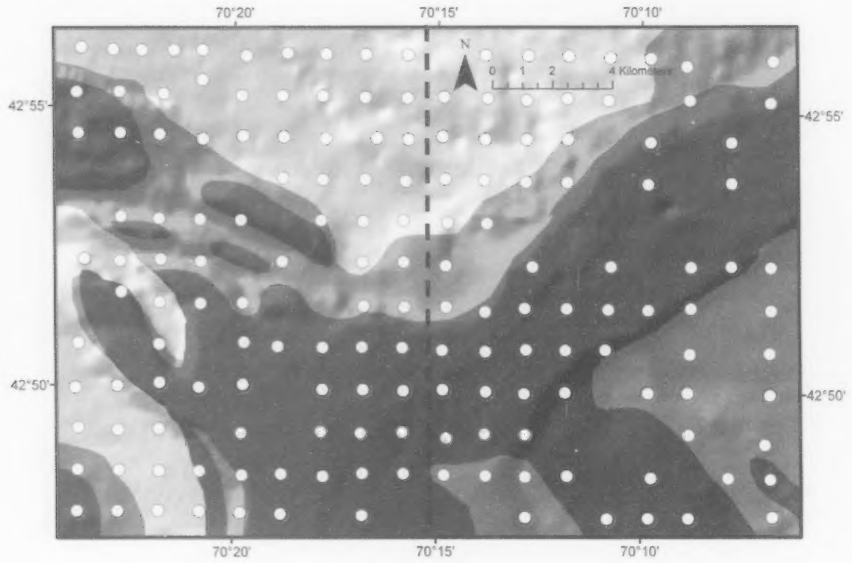


**Figure 2**

Locations of 163 seafloor sampling sites (white circles) within the 515 km$^2$ University of New Hampshire study area that were sampled by some combination of Shipek grab, Wildco box corer, and towed video camera. Distribution of three major bottom sediment types: light gray=mud; medium gray=sand; dark gray=gravel (which includes all hard bottom types ranging from gravel to boulders).Vertical dashed line is the western boundary of the WGOM closure.

latitude and 1 min of longitude), yielding a total of 216 target sampling sites (not all were sampled). A combination of techniques was used at each site, including grab and box samples for infauna and sediments, and towed video for epifauna and general seafloor conditions, as described below.

### Field and laboratory methods

All seafloor sampling occurred from June 2002 through September 2005. However, most sampling occurred during 2002 (~65% of the grab and box core sampling) and 2004 (~90% of the video sampling, and ~30% of the grab and box core sampling). Hence, 90–95% of all seafloor sampling occurred during 2002 and 2004, and there was no temporal bias in sampling inside compared to outside of the closure. In other words, approximately equal numbers of sites inside and outside of the closure were sampled during each year of the study. All sampling gear was deployed from chartered commercial fishing vessels.

Bottom sediments were sampled with either a Shipek grab (Wildlife Supply Co., Buffalo, NY) with a design sampling area of 0.04 m$^2$, or a Wildco box corer (Wildlife Supply Co.) with a design sampling area of 0.0625 m$^2$. The depth of sediment penetration was measured to the nearest 0.5 cm for each sample, and a subsample of sediment was taken for grain size and organic content analyses. Grab samples were rejected if they did not penetrate at least 2 cm into the sediment, and box core samples with less than 5 cm penetration were rejected. The contents of the box corer were subsampled with a 10.4-cm inside diameter (0.0085 m$^2$ surface area) acrylic core tube. The entire grab contents and box core subsamples were washed through a 0.5-mm mesh sieve, fixed in 3% unbuffered formalin, and preserved in 70% isopropanol. In the laboratory, all invertebrates were removed under 3× magnification, sorted by major taxa, identified to family level in most cases, counted, and weighed (wet weight of preserved specimens).

Bottom sediments were analyzed for grain-size composition (texture) by using standard sieve and pipette analytical techniques (Folk, 1980). Organic content was determined by loss-on-ignition (% LOI) after 4 hours at 450°C (Byers et al., 1978).

Epifaunal assemblages were determined from bottom videographs taken with a custom-made camera system composed of a video camera mounted on a frame with synchronized strobe lights and an integrated positioning system. At each station the camera was suspended near the bottom (within 50 cm) and 6 to 10 minutes of downward looking video footage was recorded along a drift transect at least 50 m long. For quantitative analyses, the videotape from each transect was subsampled to isolate still images of sufficient quality to characterize seafloor features so that a series of nonoverlapping

images ($n$=13 to 94 per transect) along the length of each transect was produced. Each still image was analyzed for bottom characteristics (predominant sediment type), visible burrow characteristics (size, density), and epifauna (taxa, density). A trio of laser beams fixed at known distances apart allowed the total area of each image to be determined.

### Data analysis

The major focus of this study was to determine the effects of the WGOM closure by comparing samples taken inside the closure with samples taken outside the closure. Thus, potential confounding factors (e.g., sediment type, water depth, and sediment organic content) that might have affected comparisons between samples taken inside the closure with those taken outside (hereafter referred to as "in vs. out" comparisons) were assessed. Also, for the infauna analyses, data from the two sampling devices (grab and box corer) were analyzed separately to avoid complications with different sample sizes and selectivity of the gear.

#### Infauna (sampled by grab and box corer)

For both sampling devices, the role of sediment grain size (mud, sand, or gravel), water depth, sediment organic content (% LOI), and relative penetration of the sampling device were assessed, along with the factor of primary interest: whether the sample was taken from inside or outside the closure. Separate analyses were performed to examine each of the three dependent univariate infauna community variables: 1) density, 2) biomass, and 3) taxonomic richness.

All analyses were performed by using generalized linear models (GLM; McCullagh and Nelder, 1989) in S-PLUS 7.0 (Insightful Corporation, Seattle, WA). For normally distributed data or data that could be transformed to approximate normality, significance for main effects and interactions were examined by using a combination of forward and backward model selection based on the $C_p$ statistic and sequential $F$-tests in an analysis of deviance with alpha levels of 0.05 and 0.10, respectively. For non-normally distributed data, significance for main effects and interactions were examined by using sequential chi-square tests in an analysis of deviance with alpha levels of 0.05 and 0.10, respectively. For each model, the inclusion of either Gaussian or exponential spatial correlation was examined with the extended generalized linear modeling capabilities in the S-PLUS correlated data library.

#### Epifauna (documented along video transects)

A generalized linear mixed-effects (GLME) model with Poisson error and log link was used to analyze the video count data of epifauna taxa and density (Breslow and Clayton, 1993). The mixed-effects portion of the model was necessary to account for multiple images within each transect and the images that may have been correlated. The use of GLME allowed individual transects to differ from one another for reasons unaccounted for by the data and directly accounted for the repeated-measures nature of the data. S-PLUS 7.0 and the GLME extension from the S+ Correlated Data library (vers. 1.0, release 1) were used for all analyses. The significance of each fixed-effect, both main effects and interactions, was tested in an ANOVA framework by using marginal $F$-tests (Pinheiro and Bates, 2000) based on the (restricted) penalized quasi-likelihood with an alpha-level of 0.05 for main effects and 0.10 for interaction effects (Sokal and Rohlf, 1981). In addition to the main effect of inside or outside of the closure, water depth and sediment type were assessed as potential confounding factors. Offsets were included in each model to account for the fact that the total surface area observed varied from image to image because of the variations in the height of the camera. Separate analyses were performed on the two dependent variables: total density and taxonomic richness.

## Results

Preclosure and postclosure plots of the report data from fishing vessel trips showed several relevant patterns (Fig. 3). First, both preclosure and postclosure trip data verified the general expected pattern that trawls are mainly used on soft sediments in deeper water (greater than 60 m), and gillnets are used mainly on rocky bottoms in shallower areas (less than 60 m). These patterns indicate that a major effect of the closure was the removal of trawl impacts from the deeper, mainly finer sediments, and the removal of gillnet impacts from rocky areas along the top and southeast flank of Jeffreys Ledge.

The report data indicated that total gillnet fishing intensity in the overall 515-km$^2$ study area was similar before closure (2056 trips) and after closure (1812 trips); however, gillnet intensity nearly doubled in the area outside of the closure after closure (761 trips before closure compared to 1494 trips after closure). This pattern indicates that any data interpreted as showing recovery of benthic communities in rocky areas where gillnets were the major gear type needs to be tempered because of increased postclosure gillnet fishing intensity in the "control" area outside the closure.

In contrast, trawling intensity in the overall 515-km$^2$ study area decreased from 1103 trips before closure to 581 after closure (Fig. 3). There was a 39% decrease for trawl intensity outside the closure, from 894 trips before closure to 544 trips after closure. Moreover, the only portion of the study area inside the closure that was likely strongly affected by trawls before the closure was the deeper area north of Jeffreys Ledge. This information indicates that any data interpreted as showing recovery of benthic communities in soft sediment areas needs to be tempered because of the decreased trawling intensity after closure in the control area outside the closure.

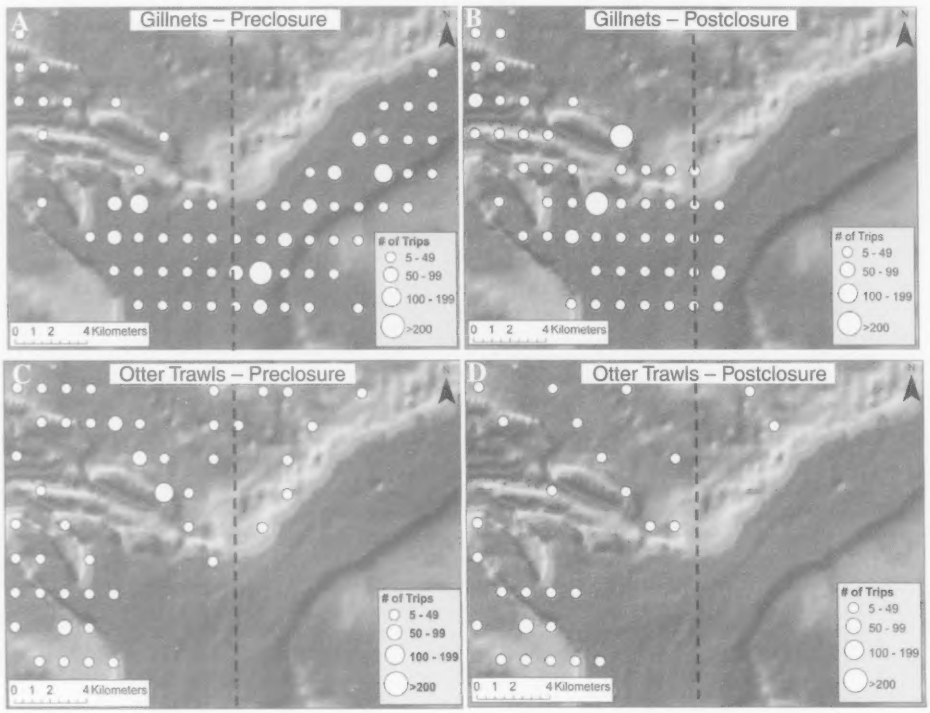


**Figure 3**

Intensity of major fishing gear activity (gillnets and otter trawls) within the study area 4.5 years before the closure was implemented (1994–98, preclosure) and 4.5 years after closure (1998–2002, postclosure) based on vessel trip report data from the National Marine Fisheries Service. Vertical dashed line is western boundary of the WGOM closure. The size of the white circles reflects the number of fishing trips (see key).

A total of 163 sites in the 515-km$^2$ study area were sampled with some combination of grab, box corer, and videotape recording (Fig. 2). Bottom types ranged from organic-rich muds in deeper water (greater than 100 m) to hard bottom (gravel and boulders) in water less than 80 m deep. Hard bottom areas typically were composed mainly of gravel and in many cases sufficient amounts of sand to allow grab samples to be successfully obtained for infauna analysis. Some hard bottom sites also had boulders present, as determined from the video imagery, and these sites were classified as gravel (Fig. 2). No plants were collected in the grab and core samples or observed in the video imagery. Infauna dominated the soft sediments in deeper waters (and were mainly sampled by box corer and video recorder), and epifauna dominated the shallower hard bottom areas (mainly sampled by grab and video recorder).

There were strong differences across the three sediment types for both infauna and epifauna. For the infauna, there were three significant sediment interaction effects for density, biomass, and taxonomic richness from grab data; all three measures were substantially higher in sand sediments inside the closure than outside the closure (Fig. 4). For the epifauna, there were significant sediment interactions for density and taxonomic richness; both variables were substantially greater only in gravel sediments inside the closure. Although the interaction effects clouded interpretation of the main effects (i.e., all sediments combined), there was consistency in that all measures were higher inside the closure compared to outside the closure. There were two significant main effects (Fig. 4, A–F): higher densities of infauna from grab ($P$=0.01) and box corer ($P$=0.02) data inside compared to outside the closure. There were two significant main effects comparisons for epifauna (Fig. 4, G–H): total community density ($P$=0.0001) and taxonomic richness ($P$=0.0004), which were both higher inside.

Overall, these data indicate the following trends by sediment type. For mud, there were no consistent trends

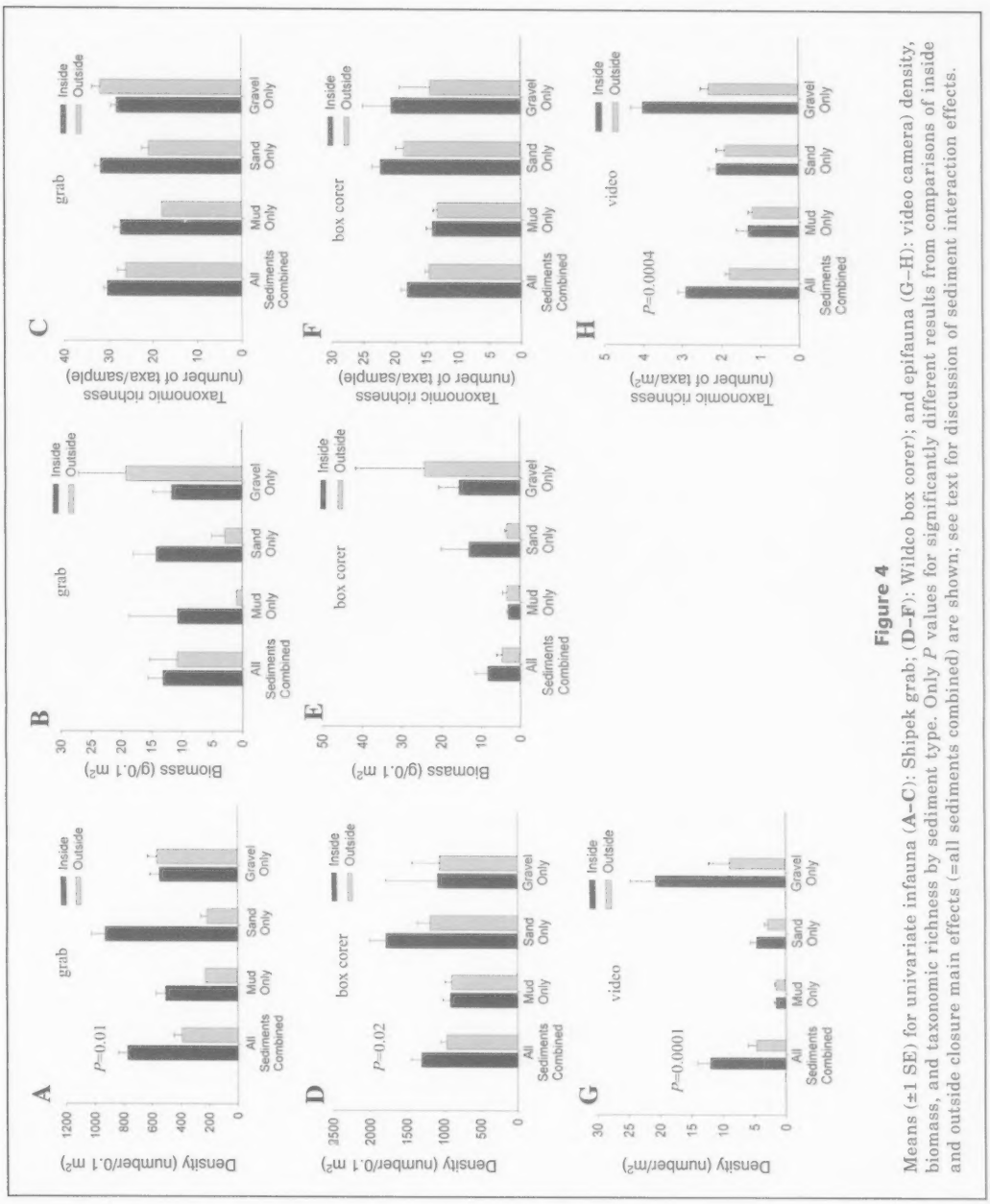


**Figure 4**

Means (±1 SE) for univariate infauna (**A–C**): Shipek grab; (**D–F**): Wildco box corer); and epifauna (G–H): video camera) density, biomass, and taxonomic richness by sediment type. Only *P* values for significantly different results from comparisons of inside and outside closure main effects (=all sediments combined) are shown; see text for discussion of sediment interaction effects.

for significant in vs. out differences for infauna or epifauna. For sand, there were strong and consistently greater density, biomass, and taxonomic richness for infauna inside the closure, but no in vs. out differences for epifauna. For gravel, there were no in vs. out differences for infauna, but greater density and taxonomic richness for epifauna inside the closure. In the context of fishing gear use, these data indicate recovery inside the closure from the negative impacts of otter trawls on infauna in sand, but not mud, and recovery inside the closure from the impacts of gillnets on epifauna on gravel bottoms. The magnitude of recovery, however, cannot be directly inferred from these data because of the confounding factor of different fishing intensities when the preclosure and postclosure data were compared (Fig. 3). The overall negative impact of trawls

(on sand sediments) was likely underestimated by our data, and the negative impact of gillnets (on gravel) was likely over estimated.

## Discussion

In control-impact studies like this one, spatial effects cannot typically unambiguously be distinguished from temporal effects of the activity of interest, because no data are available from before the activity was started (Osenberg et al., 1994). Hence, such studies (in contrast to the more rigorous before-after, control-impact [BACI] designs) must be assessed with respect to how factors that may have changed over time in relation to the control and impact areas might have contributed to any observed differences. Thus, it is possible that there were differences in the benthic communities before the WGOM closure was implemented that could have affected our interpretation of the data. Although this possibility cannot be unequivocally discarded, several lines of evidence indicate it is reasonable to assume similar conditions in both areas before the closure. First, the overall study area was chosen so that the control sites with continued fishing outside the closure were in close proximity to the treatment sites inside the closure where fishing impacts were removed; this proximity of the two areas minimized potential confounding differences related to distance. Second, the range of habitat types and relative coverage area by each type were similar inside and outside the closure. Finally, we know of no other events since establishment of the closure—other than fishing gear restrictions and subsequent intensity patterns—that may have differentially affected the study areas inside and those outside the closure. Therefore, although we feel it is reasonable to interpret the differences inside and outside the closure in our data mainly to be the result of the removal of gillnets and otter trawls from the closed area, details on the spatial distribution patterns for each type of gear use must be considered in order to fully assess these impacts.

The obvious intent behind the WGOM closure was to eliminate negative impacts from both gear types inside the closure. Implementation of the closure, however, also caused a shift in fishing intensity, particularly for gillnets. Although this shift did not affect the overall conclusion of significant impacts for both gear types and subsequent recovery inside the WGOM closure, it does indicate that the relative levels of impact of the two gear types may have been exaggerated by our data: the impacts of gillnets may have been over-estimated because of the substantially increased postclosure fishing intensity outside, and the trawl impacts may have been under-estimated. Displacement of fishing effort and intensity may be the general trend for fishing closures, and there is no straightforward way to estimate the magnitude of this effect (Ward, 2004; Fogarty and Murawski, 2005).

The major conservation concerns regarding the use of gillnets have been bycatch and entanglement of non-target species (He, 2006). Our data, however, strongly indicate that gillnets have been responsible for substantial reductions in epifauna on Jeffreys Ledge. Although Malik and Mayer (2007) reported seafloor marks on top of the ledge which may be evidence of the use of other bottom fishing gear in this area, available fishing activity data on preclosure and postclosure gear use in the study area indicate that gillnets are the major gear used on rocky bottoms in the area. Therefore, the substantial and significant differences between epifauna densities and taxonomic richness inside (compared to outside) the closure would indicate that macrofaunal communities on hard bottoms were damaged by gillnets and are recovering from these effects.

Our data from soft-sediment areas where otter trawls were the dominant gear type reflect the general trends observed in other studies (see reviews by Dorsey and Pederson, 1998; Watling and Norse, 1998; Johnson, 2002; and Kaiser et al., 2006): decreased density, biomass, and taxonomic richness in benthic communities. To our knowledge, only one previous study of bottom habitats inside the WGOM closure has been completed, an M.S thesis by Knight (2005). This research focused on areas north of our study area that were mainly affected by trawls, and it also had a similar study design of inside vs. outside a closure. The sampling occurred from 2002 through 2004, covering nearly the same time interval as our project, but also included areas in the easternmost portion of the WGOM closure that were not incorporated into the closure area until 1999 (two years after the initial closure area was implemented). These differences aside, Knight (2005) reported findings similar to ours: much higher abundances for some infaunal and epifaunal taxa inside the closure compared to outside. Knight (2005) also noted that a shift in taxonomic composition of infauna (e.g., increases in sabellid polychaetes inside the closure) and epifauna towards taxa less tolerant of physical disturbances had occurred at sites inside the closure.

As already noted, our data indicate substantial recovery but cannot be used to accurately infer the magnitude of recovery because of concurrent and confounding changes in fishing intensity during the study period. Additionally, our understanding of the recovery process for seafloor habitats disturbed by fishing gears is in the early stages—in large measure because of the number of factors involved (e.g., gear type, frequency and intensity of gear disturbances, and seafloor sediment type). Recent meta-analyses of gear effects have revealed widely variable recovery times, and no consistent trends for many combinations of gear and bottom types (Collie et al., 2000; Kaiser et al., 2006). In two recent studies on nearby Georges Bank in the Gulf of Maine few measurable effects of two large fishing closures were found for epifauna and infauna. Link et al. (2005) reported no significant differences in a variety of measures of benthic communities from video recordings and grab samples when comparing sites fished mainly by scallop dredges outside the closed areas with those inside the closures 4.5 years after closure. They attributed their

findings to the naturally dynamic nature of the seafloor that is frequently affected by storms and strong tidal currents (also see Auster and Langton, 1999; DeAlteris et al., 1999; Henry et al., 2006). Stokesbury and Harris (2006) reported similar findings for a video study of epifauna in the same general study areas. In contrast, Collie et al. (2005) showed substantial recovery of benthic epifauna (megafauna) on gravel bottoms inside one of the closed areas on Georges Bank, but differences did not occur until 2.5 years after closure, and increases in biomass and abundance of some taxa were still occurring after five years. They suggested that recovery times for faunal communities on gravel bottoms in their study area were on the order of ten years. In sum, these data further indicate that long-term studies will be needed to fully assess the effects of the WGOM closure on the recovery process for benthic communities on hard bottoms.

A similar conclusion can be drawn for recovery of benthic communities on muddy bottoms inside the closure: the recovery process is still ongoing. Previous studies that indicate otherwise, however, need to be considered. For example, two studies in the western Gulf of Maine indicated rapid recovery of mud bottom communities. Sparks-McConkey and Watling (2001) reported recovery to ambient levels for the infauna on muddy bottoms within 3.5 months after experimental trawling disturbance. Simpson and Watling (2006) also found only short-term (less than three months) effects on mud-bottom infaunal communities regularly fished by shrimp trawls with rock hopper gear compared to an adjacent unfished area. In both studies, the dominant taxa were small, nearsurface-dwelling species (mostly polychaetes) with high reproductive rates and thus potentially able to recover quickly from disturbance. It should also be noted that both these studies involved much smaller spatial scales (two study areas, each <40 $km^2$) than that of our study (515 $km^2$) such that immigration over smaller distances may have resulted in faster recovery rates. In contrast to these findings of minimal effects and fast recovery, some studies have shown substantial effects of otter trawls on mud-bottom communities and long recovery times, and the differences can in part be explained by differences in dominant taxa. If mud bottom communities include long-lived species, many of which are also structure-forming, then trawls (and other mobile gear) can have substantial adverse effects, as was found in the meta-analysis of Collie et al. (2000). Jennings et al. (2001) and Queirós et al. (2006) found significant decreases in infaunal biomass and production on muddy bottoms related to trawling intensity, as well as differential responses in relation to size spectra of the dominant taxa. Hixon and Tissot (2007) documented 600% higher densities of epibenthic invertebrates (and 23% more fish) based on video transects in untrawled (compared to trawled) mud bottom areas. Long-lived, slow-growing sea pens (*Stylatula* spp.) dominated the invertebrate communities in untrawled areas, but were rare in trawled areas. Tillin et al. (2006) reported similar sea-basin scale patterns in benthic communities in the North Sea in relation to fishing intensity; large, suspension-feeding epibenthic taxa dominated in lightly trawled areas, whereas mobile taxa and infaunal and scavenging species were dominant in areas that were more heavily trawled. Therefore, the level of effect and recovery times for benthic communities on mud bottoms affected by bottom trawls can be expected to vary widely, dependent in part on characteristics of the dominant species, and areas dominated by large or structure-forming taxa can be the most negatively affected. Further studies will be required to characterize the process over the long term, and such research should include more rigorous assessment of variations in fishing intensity than was possible in our study (Hiddink et al., 2006).

A final topic here concerns possible indirect effects on benthic communities caused by removal of fishing pressure on species that consume benthic invertebrates (Pinnegar et al., 2000). Unfortunately, little information is available on how fish populations have responded to the WGOM closure. However, available data indicate little or no increase in populations of fish such as gadids, skates, and flatfish that prey on benthic invertebrates (Murawski et al., 2004, 2005). However, recent experimental tethering studies showed greater predation rates on adult crabs (*Cancer* spp.) in rocky areas inside the closure (Meyer, 2005). Meyer also found very slow colonization rates on experimental habitat plots inside and outside of the closure, and no significant effects of predator exclusion cages on these rates. In sum, these data suggest that any indirect effects caused by differential predation rates inside compared to outside of the closure would have been weak. Meyer's (2005) colonization experiments (which were conducted over 4- to 12-month periods) also indicate that any recovery rates occurring inside the closure would be relatively slow.

The WGOM closure area is achieving a very important management goal: protection and enhancement of seafloor habitats. How are these changes related to ongoing recoveries of some fish populations and what are the management implications?

Closed area management measures are expected to have two separate effects on productivity and sustainability of fisheries (Stefansson and Rosenberg, 2005). First, if the closure is properly designed, it may reduce fishing mortality rates on some stocks by shifting limited effort away from areas where catchability is high to areas where it is lower. Effort control is essential to ensure that effort increases don't compensate for reduced catchability. Reduced fishing mortality rates on overfished stocks should increase productivity through higher stock and recruitment levels.

Secondly, closed areas that protect habitat may increase productivity of stocks by increasing growth, reproduction, and survival rates. This effect of closed areas is much harder to quantify or demonstrate with respect to recovery of the fish stocks. But, one important aspect of inferred habitat quality is the abundance of food resources for fishes, including both infauna and epifauna.

The management implications from this study are also two-fold. The observed differences in infauna and epifauna indicate that the closed area has been an effective measure for protecting habitat and potentially aiding stock recovery, although the latter has yet to be quantified. This conclusion is important regionally because closed area effects on reducing fishing mortality in New England have been generally accepted, but habitat protection benefits are still controversial.

A second important implication from this study is in the design of closed areas. The results presented here indicate that the particular bottom types that benefit most from protection depend on the type and intensity of fishing gear use before the closure is established. For example, the rocky bottoms in our study area were heavily fished with gillnets before the closure was implemented, and they showed the most recovery for epifauna, but little difference for infauna. As management of closed areas is modified in the future, these results will help clarify design criteria and refine the design of closures. Coupled with the improved ability to accurately monitor the position of vessels during fishing operations, these data may enable managers to more finely tailor spatial characteristics of closed areas thereby improving the effectiveness of management measures while potentially reducing the restrictions on fishing operations.

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# Development and growth of hatchery-reared larval Florida pompano (*Trachinotus carolinus*)

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**Abstract**—Although the Florida pompano (*Trachinotus carolinus*) is a prime candidate for aquaculture, the problematic production of juveniles remains a major impediment to commercial culture of this species. In order to improve the understanding of larval development and to refine hatchery production techniques, this study was conducted to characterize development and growth of Florida pompano from hatching through metamorphosis by using digital photography and image analysis. Newly hatched larvae were transparent and had a large, elongate yolk sac and single oil globule. The lower and upper jaws as well as the digestive tract were not fully developed at hatching. Rotifers were observed in the stomach of larvae at three days after hatching (DAH), and *Artemia* spp. were observed in the stomach of larvae at 14 DAH. Growth rates calculated from total length measurements were 0.22 ±0.04, 0.23 ±0.12, and 0.35 ±0.09 mm/d for each of the larval rearing trials. The mouth gape of larvae was 0.266 ±0.075 mm at first feeding and increased with a growth rate of 0.13 ±0.04 mm/d. Predicted values for optimal prey sizes ranged from 80 to 130 μm at 3 DAH, 160 to 267 μm at 5 DAH, and 454 to 757 μm at 10 DAH. Based on the findings of this study, a refined feeding regime was developed to provide stage- and size-specific guidelines for feeding Florida pompano larvae reared under hatchery conditions.

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Jacks and pompanos of the family Carangidae are represented by 33 genera and approximately 140 species found in tropical and subtropical oceans around the world (Helfman et al., 2003). Because carangids are highly regarded as food and game fishes, many species are exploited worldwide. Common to the southeastern Atlantic and Gulf of Mexico coasts of the United States, the Florida pompano (*Trachinotus carolinus*) is a prime candidate for aquaculture and stock enhancement programs because of its high value and limited availability from commercial harvests (Watanabe, 1995; Craig, 2000).

The larvae and early juveniles of 28 species of carangids of the South Atlantic and Gulf coasts have been described with some detail (Goode, 1882; Starks, 1911; Ginsburg, 1952; Aprieto, 1974; Laroche et al., 1984; Fahay, 2007). Fields (1962) described wild-caught Florida pompano ranging in size from 4.0 to 42.1 mm total length (TL), and more recently Fahay (2007) described fish ranging in size from 3.1 to 14.8 mm TL. Although these reports provide essential information on the early life history of jacks and pompanos for fishery biologists, additional knowledge on the morphological development, growth, and trophic requirements is essential to evaluate new species for culture and to develop effective hatchery rearing techniques and feeding regimes.

Significant interest in the culture of Florida pompano developed in the United States during the 1960s and 1970s and, as a result, efforts were initiated to develop spawning and rearing techniques for this species (Moe et al., 1968; Hoff et al., 1972, 1978a; McMaster, 1988). Florida pompano exhibit a number of desirable characteristics for aquaculture (Weirich et al., 2006). The species exhibits a high rate of growth, readily accepts commercially prepared diets, adapts to low salinity environments, and has been successfully cultured in tanks, ponds, cages, and offshore netpens. Unfortunately, the production of a reliable supply of juveniles to stock grow-out operations has been a limiting factor with respect to commercial culture of this species. Hoff et al. (1972) reported producing an average of only 300 juveniles per spawning event, and until recently, researchers and commercial culturists alike were largely unsuccessful at producing the quantities of eggs and larvae needed for commercial production or stock enhancement programs. Weirich and Riley (2007) reported that in a series of nine spawning trials conducted over a two-year period, 3.2 million fertilized eggs were produced by 40 adult Florida pompano (1:1 sex ratio). These

values approximate the necessary quantities needed for mass production. The advent of new broodstock management techniques for domestication and controlled reproduction in captivity offer great promise for the culture of Florida pompano. However, there is a need to develop and refine hatchery technologies for this species because larvae undergo major functional and morphological changes throughout their early life history.

Florida pompano eggs are typical of marine fishes with pelagic eggs. In a single spawning event, one female can produce 200,0000 to 400,000 small, buoyant eggs that range in size from 0.87 to 1.00 mm in diameter (Hoff et al., 1978a). Florida pompano eggs normally have a single oil globule, although eggs from some broodfish reportedly have several small oil globules. The size and number of oil globules within eggs can serve as an indicator of egg quality and correlate with the amount of energy available for developing larvae (Barbaro et al., 1991). The yolk that is deposited during vitellogenesis must provide nutrition for the developing embryo and larvae. Newly hatched Florida pompano larvae are approximately 2.0 mm TL and are not well developed (Hoff et al., 1978b). Depending on water temperature and developmental rates, larvae use yolk reserves for two to three days after hatching (DAH), which coincides with pigmentation of the eyes, mouth formation, and first feeding. Florida pompano larvae have been cultured by using a variety of live zooplankton, including copepods, rotifers, and *Artemia* spp. Florida pompano undergo metamorphosis at 24 DAH at 15 mm TL and can easily transition to dry feeds (McMaster, 1988).

Previously published descriptions of Florida pompano larvae provide limited details on development and growth under hatchery conditions. To improve the understanding of larval development of this species, the present study was conducted to measure the growth of larvae from hatching through metamorphosis by using digital photography and image analysis. The specific objectives were 1) to compare morphological variation among larvae from three different spawning trials; 2) to document time of occurrence for critical periods including first feeding, yolk and oil globule exhaustion, gas bladder inflation, transition in diet, and onset of metamorphosis; and 3) to develop a model feeding regime for Florida pompano larvae.

## Materials and methods

### Spawning and egg incubation

This study presents data regarding developmental characteristics and growth of larvae obtained from captive reproduction of Florida pompano broodstock held at the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) Center for Reproduction and Larviculture in Fort Pierce, Florida (Weirich and Riley, 2007). Broodstock (sex ratio, 1:1) were held in recirculating tank systems under controlled photothermal conditions and were sampled periodically to assess health and reproductive condition. To initiate spawning, ripe females (mean oocyte diameter ≥500 μm) and males were implanted with a 75-μg slow-release pellet of gonadotropin-releasing hormone analogue (Syndel International, Inc., Vancouver, BC). Fish spawned volitionally approximately 36 hours after hormone implantation, and eggs were collected and stocked into aerated 100-L incubation tanks (24–26°C). Hatching occurred approximately 30–36 hours after fertilization.

### Larval culture

In three independent larval rearing trials (initiated 15 June 2004, 17 June 2004, 17 August 2005), approximately 50,000 larvae (0 DAH) were stocked into a 1.0-$m^3$ round fiberglass tank. The tank was filled with 800 L of natural seawater that had been subjected to biological and mechanical filtration, in addition to ultraviolet sterilization, before use. Water quality was monitored daily with a multiparameter dissolved oxygen probe (YSI Incorporated, Model 85, Yellow Springs, OH). Water was not exchanged from zero DAH through five DAH. After five DAH, water quality was maintained by daily water changes ranging from 50% to 200% through 20 DAH.

Although similar production methods were used during each year of the study, trials were conducted within a greenhouse during the first year of the study and within an insulated, climate-controlled hatchery during the second year. After stocking, tanks were gently aerated and surface light levels were maintained at 300 lux (model LI-189, LI-COR, Lincoln, NE) for 16 hours daily. At two DAH, the aeration level was increased, tanks were inoculated with cultured microalgae (*Nannochloropsis oculata*) (Instant Algae, Reed Mariculture, Campbell, CA) to maintain green water culture conditions, and surface light levels were increased to 2000 or 3000 lux. Larvae were fed enriched rotifers (*Brachionus plicatilis*; 53–225 μm) from two DAH through 15 DAH. Rotifer strains and size distributions differed among years (Fig. 1). Larvae were fed *Artemia* spp. nauplii (~480 μm) from 12 DAH through 20 DAH (Embryon, INVE, Salt Lake City, UT). Live feed organisms were fed three times daily and were maintained at densities of one to three individuals per mL. Artificial feed (400–800 μm diameter particles) was offered to larvae beginning at 10 DAH (INVE NRD Micro Pellet, Salt Lake City, UT).

### Sample collection

Samples of 10 larvae were randomly collected daily from hatching through completion of metamorphosis at 20 DAH. Larvae were euthanized by brief immersion in cold seawater (4°C), placed on glass slides, and photographed by using a dissecting microscope at 4× magnification. A compound microscope at 100× magnification was used to photograph the head and mouth of each larva from 0 DAH through 5 DAH; thereafter, the head and mouth of each larva was photographed by using the dissect-

ing microscope. All larvae were photographed on their left sides in the sagittal plane, and a fine-point needle was used to position larvae as necessary. Microscopes were equipped with high-resolution digital cameras (Sony DSC-FS17, San Diego, CA), and photographs were recorded as uncompressed files in tagged image file format (TIFF) at 6 megapixels. For calibration, a 0.01-mm micrometer scale bar was photographed for each larval series and for both microscopes.

### Image analysis

Larvae and selected anatomical features were measured and analyzed by using SigmaScan Pro 5.0 image analysis software (SPSS Science, Chicago, IL). All morphometric measurements listed below were recorded to the nearest 0.001 mm and calibration errors were maintained at less than 1 $\mu$m (≤0.1% of 1 mm). The total length (TL) and standard length (SL) of larvae was measured along lines parallel to the longitudinal axis of the fish (Snyder 1983).

**Body depth**—The distance, perpendicular to the longitudinal axis of the body from the insertion of the first dorsal spine to the ventralmost point of the body. For yolksac larvae, the distance perpendicular to the longitudinal axis of the body from the dorsal crest through the midpoint of the yolk to the ventralmost point of the body.

**Head length**—The distance, parallel to the longitudinal axis of the head, from tip of snout to the edge of the operculum.

**Eye diameter**—The distance, parallel to the longitudinal axis of the head, from anteriormost to posteriormost points of bony orbit.

**Yolksac length**—The distance, parallel to longitudinal axis of the body, from anteriormost to posteriormost points of the yolk sac.

**Yolksac depth**—The distance, perpendicular to the longitudinal axis of the body, from ventralmost to dorsalmost points of yolk sac.

**Oil globule diameter**—The distance, parallel to longitudinal axis of the body, from anteriormost to posteriormost points of oil globule.

**Length of upper jaw**—The length of the premaxillae and maxillae to the point of articulation with the dorsal process of the dentary.

**Length of lower jaw**—The length of the dentary to the point of articulation with the angular and maxillae.

At the time of measurement, observations of larval development state were recorded to identify the chronological sequence of events. The following critical periods were noted when first observed: 1) hatching; 2) mouth formation; 3) body pigmentation; 4) eye formation and pigmentation; 5) stomach and digestive tract formation; 6) first feeding; 7) yolk exhaustion; 8) oil globule exhaustion; 9) diet transition from rotifers to *Artemia* spp.; and 10) metamorphosis. Completion of the larval stage and metamorphosis was defined as the point when the axial skeleton is ossified and fish acquire the anatomical and morphological characteristics of juveniles.

The relationship between TL and age; SL and age; and mouth gape and age were plotted separately for each larval rearing trial. Total and standard length data of larvae were fitted to a simple curvilinear equation (i.e., $y=a+b\times x^{0.5}$). The comparison between these plots allowed assessment of somatic growth pattern through time. Repeated-measures analysis of variance was then used to statistically compare growth rates among rearing trials. To normalize observations and stabilize the variance, data were logarithmically transformed before statistical analysis. Tukey's test was used to determine if significant differences existed among treatment means. Differences were considered significant at $P\leq0.05$. The general linear model function in SigmaStat 3.0 (SPSS Science, Chicago, IL) was used for all analyses.

Regression equations were calculated for total and standard length, yolk volume, and oil globule volume for larvae cultured from each spawn. Yolk volume was determined by using the equation for a prolate spheroid:

$$\textit{Yolk volume} = 4/3\ \pi\ [\textit{yolk-sac length}]\ [\textit{yolk-sac depth}]^2. \quad (1)$$

Oil globule volume was determined by using the equation for a sphere:

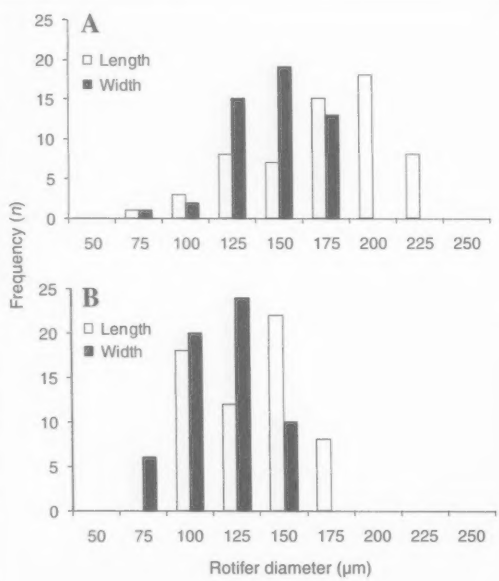


**Figure 1**

Size-frequency histograms of rotifer cultures used to feed larval Florida pompano (*Trachinotus carolinus*). Stock cultures of rotifers (*Brachionus plicatilis*) were obtained from (**A**) Aquaculture Center of the Florida Keys (ACFLK, Marathon, FL) in 2004 and (**B**) Oceans Reefs and Aquariums (ORA, Fort Pierce, FL) in 2005.

$$\textit{Oil globule volume} = 4/3\ \pi\ [\textit{oil globule diameter}/2]^3. \quad (2)$$

Length and volume data were then plotted against age. The curvilinear equation, $y = a + b \times x^{-1}$, was fitted to the yolk and oil globule volume data and plotted with 95% confidence limits.

The mouth gape was determined by using length measurements of the upper and lower jaws and the Law of Cosines equation for a triangle with two known sides and an angle between them:

$$a^2 = b^2 + c^2 - 2bc \cos \alpha, \quad (3)$$

where $a$ = mouth gape;
$b$ = upper jaw;
$c$ = lower jaw; and
$\alpha$ = angle that forms the degree of mouth opening.

Calculations were based on the assumption that during active feeding the mouth of larvae opens to an angle ranging from 90° to 120° to capture prey (Shirota, 1970). Optimal prey sizes were estimated at 30% and 50% of mouth gape for larvae (Yasuda, 1960; Shirota, 1970; Hunter and Lasker, 1981; Cunha and Planas, 1999).

## Results

During each 20-day trial, production of postmetamorphic juvenile Florida pompano ranged from 1.5 to 5.0 fish/liter. Although water temperature is an important factor governing growth, there was no significant difference among any of the water quality parameters measured among rearing trials ($P$=0.67). In the first rearing trial, dissolved oxygen was 5.7 ±0.2 mg/L (mean ± SE), temperature was 25.5 ±0.2°C, and salinity was 34.9 ± 0.6 g/L. In the second rearing trial, dissolved oxygen was 5.6 ±0.2 mg/L, temperature was 25.5 ± 0.2°C, and salinity was 34.8 ±0.5 g/L. In the third rearing trial, dissolved oxygen was 5.7 ±0.1 mg/L, temperature was 25.0 ±0.6°C, and salinity was 34.5 ±0.6 g/L.

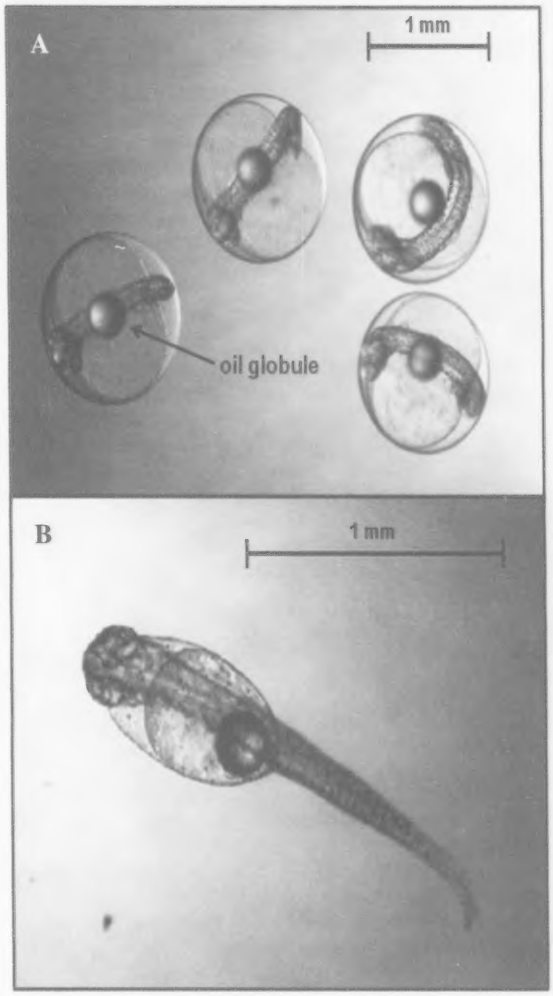


**Figure 2**
Florida pompano (*Trachinotus carolinus*) embryos at (**A**) 24 hours after fertilization, and (**B**) as yolksac larva at one day after hatching. Scale represents 1 mm.

Eggs collected from each spawning event were uniform in shape and appearance. Fertilized eggs were 0.99 ±0.04 mm in diameter and contained a single oil globule (Fig. 2A). Newly hatched larvae were transparent, small (TL=2.6 ±0.4 mm), and not well developed (Fig. 2B). As typical of carangids, larvae hatched with large, elongate yolk sacs extending beyond the head and along the ventral region of the head and gut. A single oil globule was situated at the posterior end of the yolk sac. At hatching, the lower and upper jaws, as well as the digestive tract, were not fully developed. Pigmented eyes and functional mouth parts had formed by the end of two DAH when the larval swimming pattern became stronger and feeding behavior was first observed. Melanophores were observed forming along the head and dorsal surface of the body at two DAH. The stomach and a primitive intestine were observed forming at two DAH, and the intestine had connected with the anus at three DAH. Rotifers and algae were first observed in the stomachs of larvae at three DAH. Larvae had exhausted yolk reserves at three to four DAH and the remaining oil globule at four to five DAH and were completely transitioned to exogenous feeding at five DAH (Fig. 3; Fig. 4A). Larvae at seven DAH exhibited a fully formed

and well developed digestive system (Fig. 4B). *Artemia* spp., which were first offered 12 DAH, were observed in the stomach of larvae along with rotifers at 14 DAH. Artificial feeds, which were offered beginning 10 DAH, could not be detected through observation with a dissecting microscope. Although Florida pompano lack a gas bladder, approximately 10% of larvae consumed small air bubbles (129–235 μm) that were trapped in the peritoneal cavity from 5 to 10 DAH.

Swimming ability improved and larvae were noticeably stronger with pectoral fin development at four to five DAH. The dorsal, pelvic, and anal fins began developing at nine DAH and the formation of the caudal fin and fin rays was observed at 12 DAH. Morphological and osteological development at six and seven DAH was noticeably more advanced in the third rearing trial than in specimens collected in the previous trials. As evidenced by the formation of hypural elements, notochord flexion was first observed at 10 to 12 DAH in the first two rearing trials. In the third rearing trial, notochord flexion was observed 8 to 11 DAH. Larvae completed flexion at 12 to 16 DAH in the first two rearing trials, and at 11 to 12 DAH in the third rearing trial. Postflexion and the onset of transformation were apparent at 15 to 18 DAH in the first two rearing trials (Fig. 5A), and at 13 to 14 DAH in the third rearing trial. Larvae in the first two rearing trials completed transformation and had a full complement of fins and scales by 20 DAH (Fig. 5B), whereas fish in the third rearing trial completed transformation by 17 DAH.

Differences in growth were observed among trials. Mean growth rates calculated from TL measurements were 0.22 ±0.04, 0.23 ±0.12, and 0.35 ±0.09 mm/d for each of the larval rearing trials. Larvae in the third trial grew faster than fish in the first and second trial.

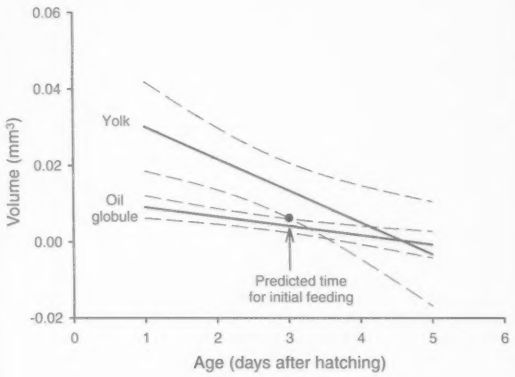


**Figure 3**

Regression (solid line) with 95% confidence limits (dashed line) of yolk volume on oil globule volume for Florida pompano (*Trachinotus carolinus*). Data represent combined measurements of three larval production trials.

Measured growth parameters of Florida pompano larvae reared from one through 20 DAH are summarized in Table 1. Statistical analysis evaluating TL and age (Fig. 6) revealed no significant differences among growth rates from hatching through six DAH ($P$=0.84); however, a significant difference in growth rate was detected for larvae reared in the third trial ($P$=0.007) after seven DAH (Table 2). No significant differences were detected between the first two rearing trials ($P$=0.12).

Similar results were observed with a statistical analysis of larvae, where SL and age (Fig. 7) were used to determine growth rates. Mean growth rates were 0.18 ±0.03, 0.18 ±0.10, and 0.31 ±0.08 mm/d for each of the larval rearing trials. No significant differences were observed among growth rates from hatch through eight DAH ($P$=0.75); however, a significant difference in growth rate was detected for larvae reared in the third

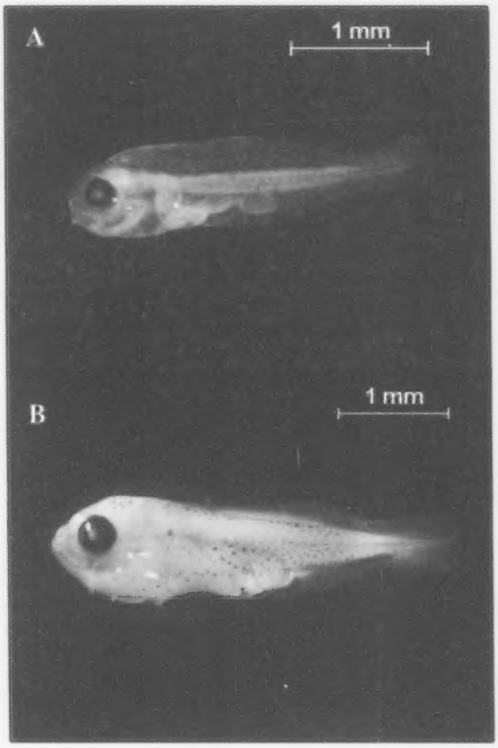


**Figure 4**

Early stage Florida pompano (*Trachinotus carolinus*) reared in the laboratory and photographed with a dissecting microscope and digital camera. (**A**) Larva at three days after hatching (DAH) near initiation of exogenous feeding and with eye pigmentation. (**B**) Larva at 7 DAH with fully formed digestive system, distinctive pigmentation, and initial development of head spines. Scale represents 1 mm.

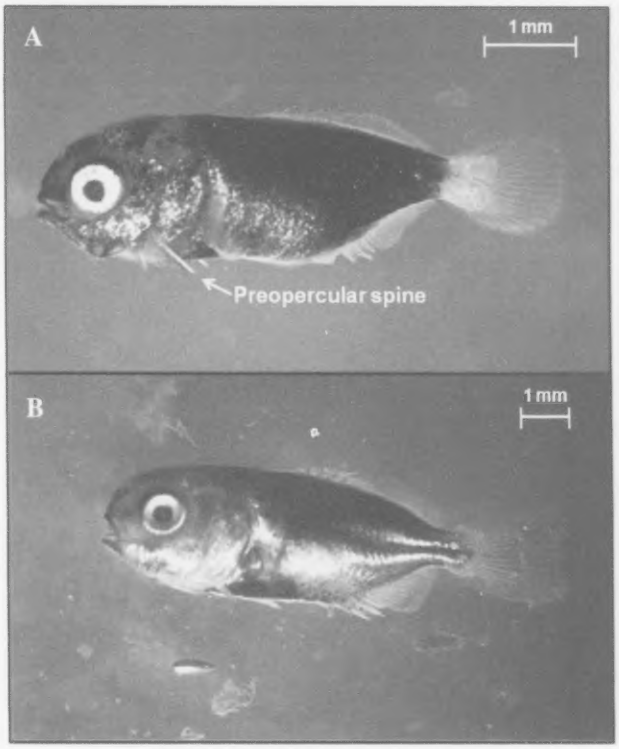


**Figure 5**

Late stage Florida pompano (*Trachinotus carolinus*) reared in the laboratory and photographed with a dissecting microscope and digital camera. (**A**) Postflexion larva at 18 days after hatching (DAH) undergoing transformation. Note presence of preopercular spines. (**B**) Juvenile at 20 DAH transitioning to artificial feeds. Scale represents 1 mm.

trial ($P<0.001$) after nine DAH. Larvae in the third trial grew significantly faster than fish in the first and second trial ($P<0.001$). No significant differences were detected among the first two rearing trials ($P=0.18$).

No significant differences were observed in larval mouth size among rearing trials ($P=0.16$). The mean mouth gape of larvae was 0.266 ±0.075 mm at first feeding and increased with a growth rate of 0.13 ±0.04 mm/d (Table 3). The length of the upper and lower jaws and mouth gape increased linearly with age (Table 4). Predicted values for optimal prey sizes ranged from 80 to 130 μm at three DAH, 160 to 267 μm at five DAH, and 454 to 757 μm at 10 DAH. These values correspond closely with the sizes of rotifers and *Artemia* **spp.** nauplii fed to larvae during each of the rearing trials.

Larvae that exhibited aggressive behavior progressed through metamorphosis earlier than their cohorts. Cannibalism, defined by Smith and Reay (1991) as the act of killing and consuming the whole, or major part, of an individual belonging to the same species regardless of its age or stage of development, was not observed in the rearing trials. The dominant, aggressive behavior observed was that of inflicting injury that resulted in mortality in small premetamorphic individuals.

## Discussion

As a direct result of aquaculture research, the larval development of a number of valuable marine fish species has been described and characterized. The early life stages of species such as striped bass (*Morone saxatilis*; Brown et al., 1998), red drum (*Scieanops ocellatus*; Lee et al., 1984), and red snapper (*Lutjanus campechanus*; Drass et al., 2000) were studied to support efforts for food production and stock enhancement in the United States, and global research on the early life history of a number of valuable species has led to the develop-

**Table 1**

Morphometrics of Florida pompano (*Trachinotus carolinus*) from one day after hatching (DAH) through 20 DAH cultured at 25°C. Metamorphosis was completed at 17–19 DAH. The relationship of head length to standard length ranged from 33% to 49%. Values are means ±standard error (SE) for larvae sampled from three rearing trials ($n$=600).

| Days after hatching | Total length (mm) | Standard length (mm) | Body depth (mm) | Head length (mm) | Eye diameter (mm) |
|---|---|---|---|---|---|
| 1 | 2.27 ±0.50 | 2.14 ± 0.58 | 0.61 ±0.06 | — | 0.19 ±0.02 |
| 2 | 2.62 ±0.31 | 2.52 ±0.50 | 0.67 ±0.10 | — | 0.25 ±0.00 |
| 3 | 2.71 ±0.27 | 2.69 ±0.54 | 0.65 ±0.04 | — | 0.28 ±0.01 |
| 4 | 2.84 ±0.76 | 2.76 ±0.25 | 0.68 ±0.06 | — | 0.28 ±0.03 |
| 5 | 3.05 ±1.01 | 2.80 ±0.25 | 0.66 ±0.07 | — | 0.29 ±0.02 |
| 6 | 3.25 ±1.26 | 2.96 ±0.24 | 0.60 ±0.07 | 1.08 ±0.19 | 0.34 ±0.17 |
| 7 | 3.55 ±1.52 | 3.06 ±0.30 | 0.74 ±0.07 | 1.17 ±0.17 | 0.31 ±0.08 |
| 8 | 3.67 ±1.50 | 3.31 ±0.51 | 0.74 ±0.03 | 1.25 ± 0.27 | 0.35 ±0.12 |
| 9 | 3.95 ±1.36 | 3.67 ±0.47 | 0.87 ±0.07 | 1.42 ±0.24 | 0.39 ±0.17 |
| 10 | 4.20 ±1.64 | 3.80 ±0.53 | 0.91 ±0.04 | 1.42 ±0.27 | 0.42 ±0.26 |
| 11 | 4.37 ±1.78 | 4.10 ±0.88 | 0.95 ±0.01 | 1.53 ±0.45 | 0.45 ±0.26 |
| 12 | 4.49 ±1.67 | 4.46 ±1.24 | 0.95 ±0.02 | 1.68 ±0.59 | 0.47 ±0.37 |
| 13 | 4.87 ±1.96 | 4.79 ±1.39 | 0.98 ±0.09 | 1.77 ±0.68 | 0.47 ±0.32 |
| 14 | 4.95 ±1.35 | 4.95 ±2.06 | 1.20 ±0.23 | 1.89 ±0.62 | 0.53 ±0.39 |
| 15 | 5.18 ±1.48 | 5.15 ±2.20 | 1.15 ±0.03 | 1.90 ±0.74 | 0.52 ±0.43 |
| 16 | 5.51 ±1.44 | 5.54 ±2.13 | 1.31 ±0.32 | 1.99 ±0.78 | 0.55 ±0.61 |
| 17 | 5.71 ±1.46 | 5.42 ±0.20 | 1.39 ±0.22 | 2.15 ±0.69 | 0.61 ±0.49 |
| 18 | 5.99 ±1.46 | 5.48 ±0.17 | 1.51 ±0.05 | 2.22 ±0.74 | 0.59 ±0.40 |
| 19 | 6.72 ±1.44 | 5.92 ±0.11 | 1.61 ±0.10 | 2.23 ±0.94 | 0.60 ±0.47 |
| 20 | 8.55 ±1.80 | 6.92 ±1.88 | 2.53 ±0.85 | 2.59 ±0.86 | 0.79 ±0.50 |

ment of standardized hatchery practices to support the growth of commercial aquaculture. Examples of such species include barramundi (*Lates calcarifer*; Kohno et al., 1986), milkfish (*Chanos chanos*; Kohno et al., 1996), mangrove red snapper (*L. argentimaculatus*; Doi et al., 1997), gilthead seabream (*Sparus auratus*; Polo et al., 1992) and European sea bass (*Dicentrarchus labrax*; Kuzir et al., 2004).

**Table 2**

Growth equations based on total length (TL) and standard length (SL) for Florida pompano (*Trachinotus carolinus*) reared at 25°C. Larval lengths were measured in mm and age was measured as number of days after hatching. Separate growth equations were developed for each rearing trial because significant differences in growth were observed ($P$<0.001).

| Trial | Equation | $n$ | $r^2$ |
|---|---|---|---|
| 1 | $TL=2.058e^{0.0533\,Age}$ | 200 | 0.96 |
| | $SL=1.967e^{0.0479\,Age}$ | 200 | 0.98 |
| 2 | $TL=2.066e^{0.9438\,Age}$ | 200 | 0.94 |
| | $SL=1.893e^{0.0508\,Age}$ | 200 | 0.93 |
| 3 | $TL=2.381e^{0.0672\,Age}$ | 200 | 0.98 |
| | $SL=2.859e^{0.0697\,Age}$ | 200 | 0.96 |

In this study, image analysis proved to be a useful tool (with a high degree of accuracy) for documenting the early development of larvae and for obtaining measurements of larval characters. The process of digitally photographing larvae and measuring growth was relatively quick and uncomplicated. Measurements were accomplished in real-time during rearing trials and therefore offered a broad spectrum of possible applications for research or commercial hatcheries. With the exception of overall growth measured as TL and SL, no substantial amount of morphological variation was expressed among cohorts of larvae from an individual spawning event or multiple spawnings. Developmental characteristics of cultured larvae were not significantly different from previous descriptions of wild-caught Florida pompano larvae from 7.2 to 11.0 mm SL (Fields, 1962); however, it was determined that fish changed morphologically from larvae to juveniles with full finray counts at 17 to 19 DAH, instead of at a previously observed time in which metamorphosis of cultured fish occurred at 24 DAH (McMaster, 1988).

Similar to the challenges in rearing any marine fish species with small eggs and larvae, the culture of Florida pompano larvae is difficult and time consuming. It is unfortunate that larvae will not readily consume

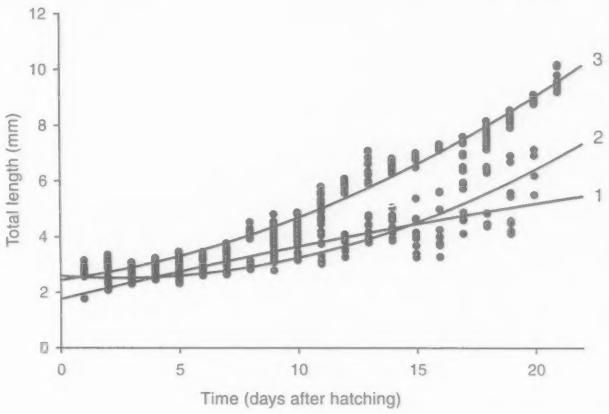


**Figure 6**

Back-calculated total length at age for hatchery-reared Florida pompano (*Trachinotus carolinus*) larvae collected from three spawnings. Each curve represents measurements collected from 200 fish. Larvae from the third trial were significantly larger after seven days ($P$=0.007).

artificial feed or *Artemia* spp. at first feeding. In nature, larval Florida pompano prey upon a wide variety of different types and sizes of zooplankton. Larvae have small mouths with limited yolk reserves and undeveloped digestive systems at first feeding. As a consequence, larvae require small, slow-moving prey that are recognizable as potential food items. Given that the optimal prey size for marine fish larvae is 25% of mouth gape at first feeding and increases to 50% within a few days (Hunter and Lasker, 1981), the production of appropriate size live feeds must be considered an essential component of larviculture protocols.

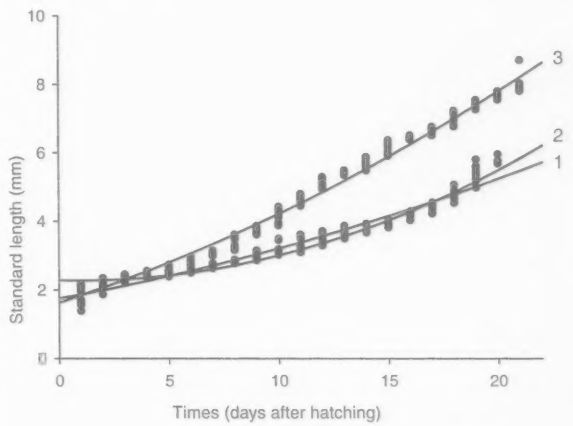


**Figure 7**

Back-calculated standard length at age for hatchery-reared Florida pompano (*Trachinotus carolinus*) larvae collected from three spawnings. Each curve represents data collected from 200 fish. Larvae from the third trial were significantly larger after nine days ($P$<0.001).

The marine rotifer, *B. plicatilis* (so-called large or small morphotypes), is the most commonly cultured and mass produced species of zooplankton worldwide (Yoshimura et al., 1996; Lubzens et al., 2001). Recent studies have shown that the strain and morphotypes of rotifer stocks within a hatchery can vary greatly, and rotifer stocks from commercial hatcheries often represent a mixture of species, strains, and morphotypes (Papkostas, 2006). Commercial hatcheries frequently buy or trade rotifer cultures with other hatcheries to meet production quotas, which can exceed one billion rotifers per day (Lubzens, 2001). In this study, we used image analysis to document the size distribution of rotifers obtained from local hatcheries. Image analysis coupled with routine sampling allowed us to monitor growth, reproduction, and size-frequency distribution of rotifer stocks. Determination of rotifer size-frequency distributions was useful for ensuring that a sufficient number of small individuals were available for first feeding larvae. Although rotifer strains and size distributions differed among years, there is little evidence that larval growth and survival was affected. Although production methods for marine rotifers and *Artemia* spp. are currently

**Table 3**

Mouth size of Florida pompano (*Trachinotus carolinus*) at the onset of opening (three days after hatching, DAH) through 20 DAH. Values represent measurements (means ±standard error [SE]) for larvae sampled from three rearing trials. Mouth gape estimates were based upon calculations assuming the mouth opens 90° (min) to 120° (max) during feeding and prey capture.

| Days after hatching | Upper jaw length (mm) | Lower jaw length (mm) | Min mouthgape (mm) | Max mouthgape (mm) |
|---|---|---|---|---|
| 3 | 0.28 ±0.02 | 0.24 ±0.00 | 0.213 | 0.319 |
| 4 | 0.29 ±0.02 | 0.27 ±0.05 | 0.250 | 0.372 |
| 5 | 0.30 ±0.01 | 0.28 ±0.05 | 0.427 | 0.640 |
| 6 | 0.36 ±0.09 | 0.38 ±0.09 | 0.525 | 0.784 |
| 7 | 0.37 ±0.12 | 0.39 ±0.19 | 0.618 | 0.924 |
| 8 | 0.39 ±0.04 | 0.43 ±0.12 | 0.763 | 1.132 |
| 9 | 0.47 ±0.11 | 0.52 ±0.16 | 1.065 | 1.550 |
| 10 | 0.53 ±0.17 | 0.59 ±0.26 | 1.221 | 1.805 |
| 11 | 0.55 ±0.02 | 0.62 ±0.22 | 1.912 | 2.838 |
| 12 | 0.61 ±0.03 | 0.68 ±0.29 | 2.265 | 3.392 |
| 13 | 0.70 ±0.03 | 0.69 ±0.35 | 3.250 | 4.863 |
| 14 | 0.77 ±0.09 | 0.73 ±0.45 | 3.472 | 5.197 |
| 15 | 0.79 ±0.03 | 0.75 ±0.39 | 4.002 | 5.979 |
| 16 | 0.88 ±0.13 | 0.85 ±0.39 | 4.383 | 6.575 |
| 17 | 0.89 ±0.07 | 0.86 ±0.50 | 5.216 | 7.757 |
| 18 | 0.94 ±0.06 | 0.89 ±0.50 | 5.306 | 7.958 |
| 19 | 1.01 ±0.18 | 0.89 ±0.62 | 5.501 | 8.252 |
| 20 | 1.06 ±0.19 | 1.03 ±0.55 | 5.878 | 8.802 |

the standard for commercial hatcheries, a variety of small copepods, protozoans, cladocerans, and molluscan larvae offer great promise as feed, provided they offer adequate nutrition.

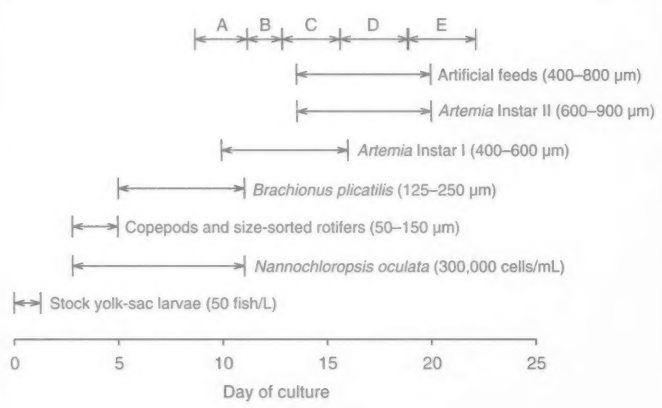


**Figure 8**

Recommended hatchery feeding regimen for rearing Florida pompano (*Trachinotus carolinus*) from hatching through transformation. Chronological stages of development are noted by the upper bar: A–preflexion; B–flexion; C–postflexion; D–metamorphosis, and E–juvenile.

As a result of the findings of this study a refined feeding regime for Florida pompano was developed (Fig. 8). The regime directly addresses the importance of feeding small prey items (80–130 µm) to larvae at first feeding (three DAH), and it provides a stage- and size-specific guideline for feeding larvae reared under laboratory conditions at 25°C. Future work should address the bioenergetics and nutritional requirements specific for Florida pompano larvae and determine the effect of increased temperature on larval growth and development. Although research conducted in this study was performed within a small-scale marine hatchery, the techniques could be used to formulate commercial hatchery production protocols for other subtropical and tropical marine species with similar early life-history patterns.

## Acknowledgments

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**Table 4**

Growth equations for upper jaw, lower jaw, and mouth gape of Florida pompano (*Trachinotus carolinus*) reared from hatching through metamorphosis. Jaw length and mouth gape were measured in mm and age was measured as days after hatching.

| Equation | | $n$ | $r^2$ |
|---|---|---|---|
| *Upper jaw* | $= 0.0492\,Age + 0.0562$ | 510 | 0.98 |
| *Lower jaw* | $= 0.0447\,Age + 0.1452$ | 510 | 0.96 |
| *Mouth gape* | $= 0.0367\,Age - 2.0308$ | 510 | 0.92 |

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# Use of non-natal estuaries by migratory striped bass (*Morone saxatilis*) in summer

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**Abstract**—For most migratory fish, little is known about the location and size of foraging areas or how long individuals remain in foraging areas, even though these attributes may affect their growth, survival, and impact on local prey. We tested whether striped bass (*Morone saxatilis* Walbaum), found in Massachusetts in summer, were migratory, how long they stayed in non-natal estuaries, whether observed spatial patterns differed from random model predictions, whether fish returned to the same area across multiple years, and whether fishing effort could explain recapture patterns. Anchor tags were attached to striped bass that were caught and released in Massachusetts in 1999 and 2000, and recaptured between 1999 and 2007. In fall, tagged striped bass were caught south of where they were released in summer, confirming that fish were coastal migrants. In the first summer, 77% and 100% of the recaptured fish in the Great Marsh and along the Massachusetts coast, respectively, were caught in the same place where they were released. About two thirds of all fish recaptured near where they were released were caught 2–7 years after tagging. Our study shows that smaller (400–500 mm total length) striped bass migrate hundreds of kilometers along the Atlantic Ocean coast, cease their mobile lifestyle in summer when they use a relatively localized area for foraging (<20 $km^2$), and return to these same foraging areas in subsequent years.

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For most migratory fish species, little is known about the size of their feeding areas, the duration of time that individuals spend there, and whether fish return to the same feeding area year after year. Migration is an essential part of the life history and ecological niche of many taxa and has been observed in marine, freshwater, and diadromous fish (Thorrold et al., 2001; Dingle and Drake, 2007). Many fish migrate between some combination of spawning area, feeding area, and overwintering area (Harden Jones, 1968; Robichaud and Rose, 2001). Many anadromous fish return to natal systems for spawning (Klemetsen et al., 2003; Quinn and Myers, 2004), but a return to feeding areas has been shown for only a few fish species (Buzby and Deegan, 2000; Solmundsson et al., 2005). Consequently, an important but unresolved issue is the extent to which individuals migrate to the same specific, non-natal, feeding area.

Populations of striped bass (*Morone saxatilis* Walbaum) exhibit a variety of movement patterns. Coastal populations at the northern and southern end of their range may move offshore from their natal estuaries in summer but do not migrate north and south along the coast (Collette and Klein-MacPhee, 2002). The U.S. Atlantic coast striped bass stocks spawn in the Chesapeake Bay, Delaware River, and the Hudson River. In the spring, some members of these stocks migrate northward along the coast to New England (Maine; New Hampshire; Massachusetts; Rhode Island; and Connecticut) and then return south in the fall (Berggren and Lieberman, 1978; Dorazio et al., 1994). Coastal migrants are caught seasonally in fisheries off all U.S. states between Chesapeake Bay and Canada (Boreman and Lewis 1987, Rulifson et al. 2008). Many of these fish are caught by hook and line, a method that targets feeding fish. These captures indicate that a wide range of possible foraging areas exist for coastal migrants. Although the spawning locations of migratory striped bass, the basic directions of coastal movements, and some size and sex-specific char-

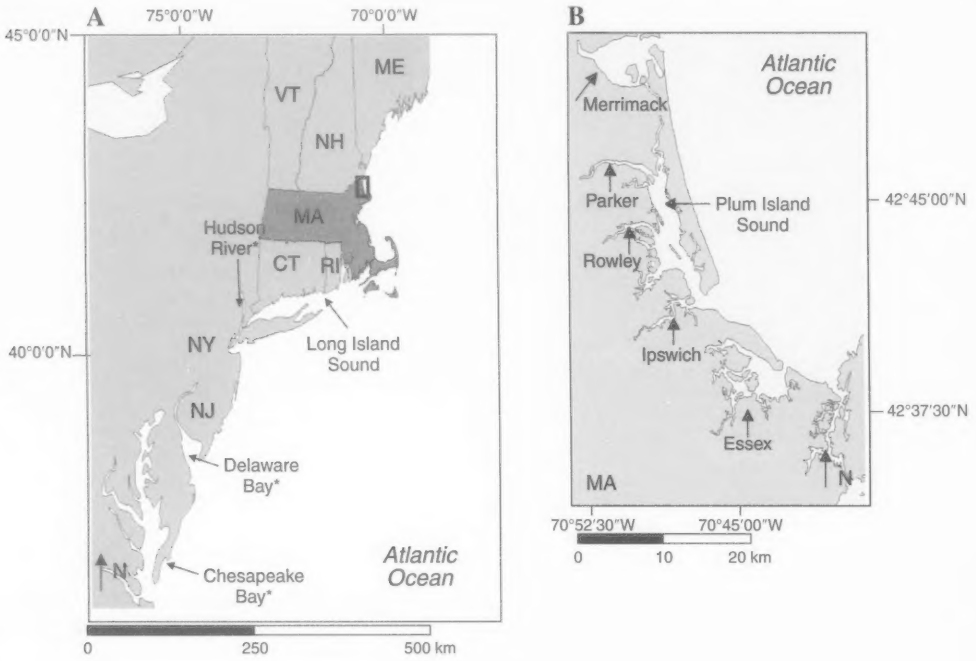


**Figure 1**

(**A**) Spawning areas and northern range of U.S. Atlantic coast striped bass (*Morone saxatilis*), the latter of which represents the largest scale by which recaptures were grouped (Massachusetts, MA). The box delineates the Great Marsh (GM). Asterisks show major spawning areas. (**B**) The Great Marsh in northern Massachusetts consists of the Merrimack, Parker, Rowley, Ipswich, and Essex river estuaries and represents the smaller scale by which recaptures were grouped. The arrangement of estuaries in the Great Marsh served as the basis for random model 2 (RM-2).

acteristics of migrants are known, regional patterns of coastal migration related to the use of specific summer areas by individual fish have not been identified.

Migratory fish that are present in non-natal estuaries during the nonbreeding (summer, fall) and nonoverwintering seasons are most likely feeding. Although the migratory stock of striped bass is widely distributed throughout New England in summer, the specific feeding areas for individual fish are not known. Migratory striped bass can stay and forage in a specific estuary that they encounter during migration or they may continue to move along, feeding in multiple estuaries for short periods. They also may either return to the site where they spent the previous summer or choose a different site each year from the many estuaries they encounter. We tested whether striped bass found in Massachusetts estuaries in summer migrated, how long they stayed in the non-natal estuaries where they were tagged, whether observed spatial patterns differed from the predictions of random models, whether fish returned to the same area over multiple years, and whether fishing effort alone could explain the recapture pattern.

## Materials and methods

From June 1999 through November 2000, 1939 striped bass (3–5 yr old, predominately the 1996 year class) were tagged with internal anchor tags. All fish (mean total length [TL]=442 mm, standard error [SE]=7.0 mm; mean wet weight=0.91 kg, SE=0.05 kg) were caught, tagged, and immediately released along the Massachusetts coast, excluding Cape Cod (Fig. 1A). The specific estuary in which fish were tagged and released, date of tagging, total length, wet weight, and tag number of fish were recorded. Several tagging (Parker, Rowley, and Essex river estuaries) and recapture sites (Merrimack and Ipswich river estuaries; Fig. 1B) are part of the Great Marsh barrier beach dune and salt marsh estuary that includes 10,117 ha of contiguous salt marsh on the North Shore of Massachusetts.

In 1999–2007, anglers voluntarily returned tags with recapture data to the U.S. Fish and Wildlife Service (USFWS) Cooperative Striped Bass Tagging Program. These recapture data included tag number, recapture date, recapture location (state, town), and approximate size of fish. Because the anchor tag was removed as

part of the reporting procedure, for most individuals, only a single recapture record existed. An approximate location (latitude, longitude, ±4 km) was created by assigning the recapture to the center of the nearest water body. When more information was included (e.g., mouth of the Merrimack River estuary), that datum was assigned a more specific recapture location. Most recapture records referenced specific locations and therefore there was relatively little error in estimating recapture location this way. Because most fish were recaptured by recreational anglers, tag reporting rate was likely similar across recapture locations.

By comparing release and recapture locations, we examined whether striped bass tagged in Massachusetts were part of the coastal migratory stock, whether they stayed in a localized area for a prolonged period in summer, and whether they returned to the same location after several years. To confirm the migratory status of striped bass tagged in Massachusetts, the location where tagged fish were recaptured in late fall, winter, or early spring was compared to the release location. To determine if striped bass remained in the same area throughout the summer, recapture locations in summer were compared to the location where fish were released. For this, two nonexclusive, spatial recapture scales were used: the larger Massachusetts coast area (MA; Fig. 1A) and the smaller Great Marsh area (GM; Fig. 1B). Two time periods were considered: the first season in which they were tagged (≤104 days and before 21 September, i.e., summer), and all times combined. Without extensive movement records on individual fish, the possibility that tagged fish moved out of the release estuary in the summer and then returned there in the fall cannot be discounted. However, to minimize this possibility, recapture records from the early (May 1 through 10 June) and late migration (22 September through 31 November) periods were excluded because these were times when migratory striped bass were hypothesized to be in transit. To determine if migratory striped bass returned to the same area in subsequent years, the number of fish that were recaptured in the area in which they were released was quantified for recaptures that occurred >12 months after release.

To examine whether the number of striped bass recaptured in their release location was different than expected by random movement models, simple, discrete time, stochastic Markov chain models were used (Agresti, 2002). These were parameterized by a series of model states (locations in the estuary or ocean where migratory striped bass could occur) connected by transition probabilities (rates at which striped bass may move between these geographic locations). Although other models have been used for animal movement, especially when large amounts of telemetry data are available (Jonsen et al., 2003; 2006), Markov chains are simple, require the least amount of data, and have been used to model biological processes (Shull, 2001; Steel et al. 2001) including movement (Hestbeck et al., 1991; Johnson et al., 2004). Furthermore, Markov chains require few assumptions; for example, all that is needed to predict the next location of an animal with this approach is knowledge of the animal's present location.

Small-scale models were used to address how many recaptures would be expected at two scales of release (MA, GM) if striped bass movements were random. Although many movement models were plausible, the examples below provide insights into how to interpret observational recapture data for migratory striped bass. In random model 1 (RM-1), model states represented three localized, geographic locations in which a feeding, migratory striped bass could be found: 1) the target or release area ($A_t$ at two scales, GM, MA); 2) the ocean; or 3) another adjacent area ($A_o$) (Fig. 2A). The probability of staying in the release or target area was $p_e$, the probability of leaving that area was $1-p_e$. In the first random model (RM-1), a fish must move through the ocean to get to another location. In RM-1, the probability of staying in the ocean ($p_o$) was 0.50, and the probabilities of staying in the two non-ocean estuaries were the same, although not necessarily 0.50 (RM-1; Fig. 2A). An assumption of RM-1 was that the fish did not prefer the release area over the adjacent area and that fish were equally likely to stay in the ocean or go to an estuary. A weekly time step was used. Transition probabilities for a striped bass in a model state always summed to 1.0.

In random model 2 (RM-2), eight states were used to simulate the complexity of the Great Marsh (Figs. 1B and 2B). Four estuarine areas ($A_1$–$A_4$) had direct connections to Plum Island Sound and represented the Merrimack ($A_1$), Parker ($A_2$), Rowley ($A_3$), and Ipswich ($A_4$) estuaries. Three of these ($A_2$–$A_4$) were connected to the ocean through Plum Island Sound whereas the Merrimack River estuary ($A_1$) was also connected directly to the ocean. The Essex River ($A_5$) was adjacent to Plum Island Sound, connected to the ocean, but not directly connected to Plum Island Sound. Neighboring estuaries that were not part of the Great Marsh were represented by ($A_o$)

Both models began with the release of 100 striped bass (individuals or schools) from the target area ($A_t$ for RM-1, or $A_3$ for RM-2) and continued until the numbers of migratory striped bass in each model state stabilized (10 weeks). The outcome predicted what proportion of model fish would be recaptured in the release estuary if movements in all directions were equally likely, i.e., random. $P_e$, the proportion of fish still in the release area after 10 weeks, was adjusted to fit observed recapture proportion data for the first summer (≤104 days and before 21 September). This weekly probability of fish remaining in the tagging estuary was matched against the observed recapture proportion. The observed recaptures for the Great Marsh were fitted to both models; the observed recaptures for Massachusetts were fitted only to the first, general model. Fitting to recaptures was possible because the model had only one parameter, $p_e$. Density dependence and intraspecific interactions were not included in these simple models. Observed and expected were compared by using $\chi^2$.

Equal catchability and effort were assumed because anglers made most recaptures and angler effort per mile of coastline was similar. This assumption allowed us to use a simpler model than the complex model of Hilborn (1990), which assumed unequal capture probabilities.

To test whether the recapture rate in the first summer after tagging was explained by patterns of fishing effort, data from the Marine Recreational Fisheries Statistics Survey were used.[1] Effort data were obtained for five bimonthly periods (March–April, May–June, July–August, September–October, November–December) in 1999 and 2000, the two years that fish were tagged. For the Coastal Household Telephone Survey (CHTS), fishing effort data were obtained from shore and private-boat anglers who live in coastal counties. Correction factors, derived from an intercept survey, were used to account for trips taken by noncoastal residents, out-of-state anglers, and anglers who live in households without telephones. Data collection occurred during a two-week period at the end of each two-month sample period. For the CHTS, a computer-assisted, random digit dialing (RDD) approach was used to contact full-time residential households who were screened to determine if any household members participated in marine recreational fishing during the previous two months. Each active angler was asked to recall the number of saltwater fishing trips that were taken during the bimonthly period, as well as asked to provide details about each trip. Institutional housing, businesses, wireless phones, and pay phones were excluded from the survey. Within each state, samples were allocated among coastal counties in proportion to household populations. For each coastal county, data from the CHTS were used to estimate the average number of trips per household and then expanded by the county household population to estimate total trips. County estimates were summed and then expanded by intercept survey adjustment factors to produce state-level effort estimates. Private-boat and shore-angler modes for trips directed towards striped bass were combined for each New England state (Maine, New Hampshire, Massachusetts, Rhode Island, and Connecticut). To compare effort expended for striped bass across states, these effort estimates were divided by km of shoreline.[2] To assess whether recaptures in Massachusetts mirrored general trends in fishing effort, the calendar dates when striped bass were recaptured in the Great Marsh and Massachusetts were compared to the timing of fishing effort. To test whether striped bass were recaptured in Massachusetts in summer, because fishing effort did not exist elsewhere in New England, bimonthly patterns of recaptures were related to summer effort scaled by coastline for all five New England states by using a Spearman correlation.

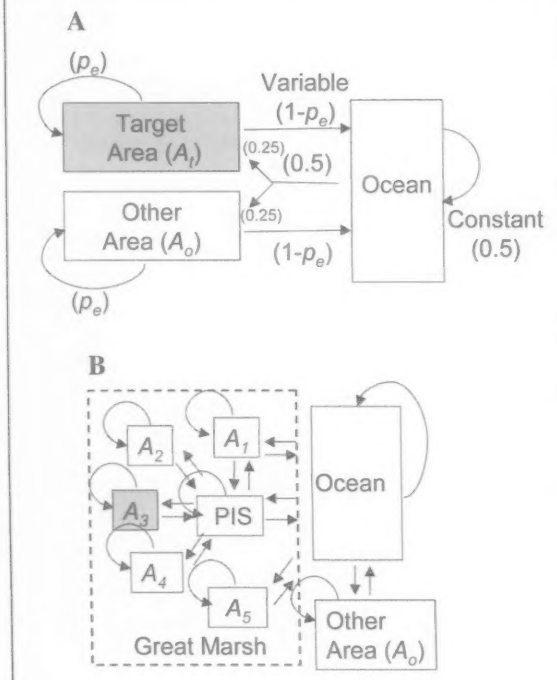


**Figure 2**

Structure of two random models used to compare observed recaptures of striped bass (*Morone saxatilis*) to what might be expected if movements into and out of the release areas were random. For all, $p_e$ was the weekly probability of a striped bass remaining in a patch and $1-p_e$ is the weekly probability of leaving. (**A**) In random model 1 (RM-1), the probability of leaving the ocean patch is 0.5 for each weekly time step. The probability of leaving the other two patches is the same but can differ from 0.5. In the comparison of the model output to recaptures, the target area may have represented either the Great Marsh or Massachusetts coast. (**B**) In random model 2 (RM-2), the complex structure represented by the Great Marsh is diagrammed: $A_1$=Merrimack; $A_2$=Parker; $A_3$=Rowley (target); $A_4$=Ipswich; $A_5$=Essex. In RM-2, the probability of remaining in all patches is $p_e$. The probability of leaving $A_2$, $A_3$, $A_4$, $A_5$ and $A_o$ is $1-p_e$. The probability of leaving $A_1$ for Plum Island Sound (PIS) or the ocean is $(1-p_e)/2$). The probability of leaving PIS for any other location is $(1-p_e)/5$). The probability of leaving the ocean for one of the four neighboring patches is (0.5/4).

## Results

Striped bass recaptures were distributed along the Atlantic Ocean coast from Maine to the Chesapeake

[1] Van Voorhees, David. Personal commun. 2000. National Marine Fisheries Service, Fisheries Statistics Division, Silver Spring, MD.

[2] Millhouser, W. C., J. McDonough, J. P. Tolson. 1998. Personal Commun. Report to National Oceanic and Atmospheric Administration, Managing Coastal Resources. 1315 East West Highway, Silver Spring, Maryland 20910.

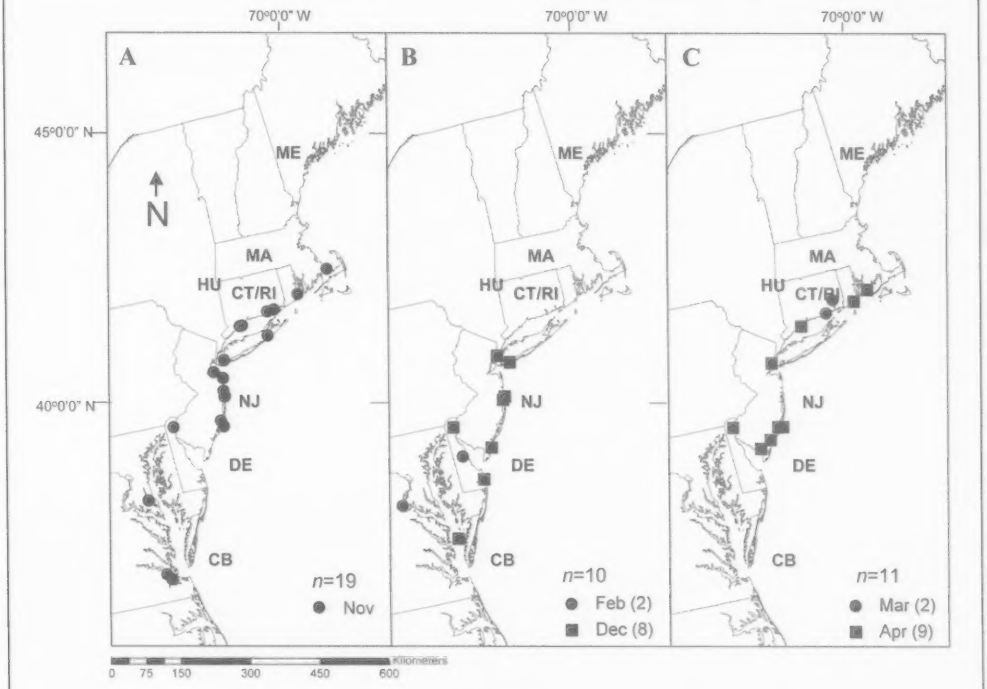


**Figure 3**

Seasonal recapture locations for all striped bass (*Morone saxatilis*) tagged in 1999 and 2000 in Massachusetts estuaries ($n$=198). Recaptures for 1999–2007 were plotted for (**A**) November, (**B**) December–February, (**C**) March–April. Because of symbol overlay, not all points are visible. ME=Maine, MA=Massachusetts, CT/RI=Connecticut and Rhode Island, HU=Hudson River (a spawning location), NJ=New Jersey, DE=Delaware River (a spawning location), CB=Chesapeake Bay (a spawning location). All years were combined. Numbers in all panels in Figure 3 and 4 sum to the total number of fish for which release and recapture locations ($n$=198) were available. Months are grouped to illustrate seasonal distributional patterns of striped bass. Fish were recaptured by angling. The map projection is Albers Equal Area Conic, NAD (North American Datum), 1983.

Bay (Figs. 3 and 4; $n$=198), indicating that striped bass tagged in Massachusetts were migratory fish. Without exception, striped bass recaptured in the late fall, winter, and early spring (November–April) were recaptured south of where they were initially tagged and released (Fig. 3, A–C). In November (end of migration), December–February (winter), and March–April (beginning of migration), with two exceptions, all recaptures occurred south of Massachusetts. For the two exceptions still in Massachusetts waters, the fish were recaptured south of where they were released. Most recaptures were made in the spring and summer when the sport fishery was most active (Fig. 4, A and B). In May and June, recaptures for all years were distributed throughout the migratory range of striped bass from New Jersey to Maine (Fig. 4A). During July, August, and September, most tagged fish, across all years, were recaptured in Massachusetts (Fig. 4B). In October, during the fall migration, striped bass were captured both within and south of Massachusetts (Fig. 4C). Fish recaptured in Massachusetts in October were caught south of the location where they were released.

Of the fish that were recaptured in the summer of the year they were tagged ($n$=41, tag to recapture ≤104 days), most stayed where they were tagged. For example, 77% (17 of 22) of the striped bass recaptured in the Great Marsh in the first summer were released there (Table 1). The Merrimack River, the northernmost river in the Great Marsh, appeared to attract striped bass with 9% of the recaptures made there even though this estuary was not a release site. All first summer recaptures were taken along the Massachusetts coast (Table 1). No striped bass tagged in Massachusetts were recaptured in any other New England state (ME, NH, RI, CT) in the first summer after tagging (Table 1).

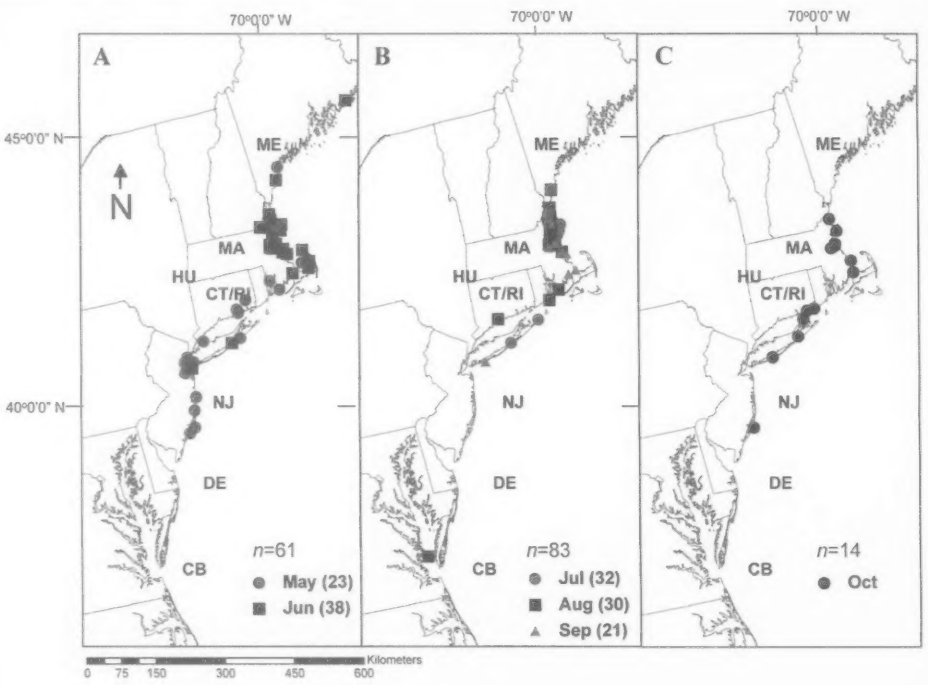


**Figure 4**

Seasonal recapture locations for all striped bass (*Morone saxatilis*) tagged in 1999 and 2000 in Massachusetts estuaries ($n$=198). Recaptures for 1999–2007 were plotted for (**A**) May–June, (**B**) July, August, September, and (**C**) October. Because of symbol overlay, not all points are visible. ME=Maine, MA=Massachusetts, CT/RI=Connecticut and Rhode Island, HU=Hudson River (a spawning location), NJ=New Jersey, DE=Delaware River (a spawning location), CB=Chesapeake Bay (a spawning location). All years were combined. Numbers in all panels of Figures 3 and 4 sum to the total number of fish for which release and recapture locations ($n$=198) were available. Months are grouped to illustrate seasonal distributional patterns of striped bass. Fish were recaptured by angling. The map projection is Albers Equal Area Conic, NAD (North American Datum), 1983.

The summer locations of striped bass along the Massachusetts coast and in the Great Marsh were not the result of random movement. If fish were choosing either the ocean or the estuary randomly, the first model predicted that only 0.25 of the tagged striped bass would be present in the release location during the first summer. This is significantly less than the proportion observed in both the Great Marsh and along the Massachusetts coast (GM, 0.77, $\chi^2$=74.6, df=2, $P$=0.005; MA, 1.0, $\chi^2$=151, df=2, $P$=0.005). The model can be used to back calculate the required probability that striped bass remained in the estuary by adjusting $p_e$ until the model output matches the observed proportion of observed recaptures. Based on RM-1, this approach indicated that to obtain the observed proportion of recaptures (0.77) for striped bass released and recaptured in the Great Marsh, the weekly probability that a striped bass remained in the Great Marsh was high ($p_e$=0.95; Table 2). Similarly, in order for all the observed recaptures (1.0) to have occurred in Massachusetts during the first summer, the weekly probability of remaining in waters off the Massachusetts coast was very high, ($p_e$=1.0; Table 2). Using the more spatially complex and realistic model (RM-2), to obtain the observed proportion of recaptures (0.77) in the Great Marsh, we found that the weekly probability of striped bass remaining in the Great Marsh was again high ($p_e$=0.75; RM-2, Table 2).

Many fish were caught where they had been released in subsequent years, and this finding would indicate that these fish return to non-natal estuaries. Across all years and times of year, 38% (41 of 108) of the recaptured fish released in the Great Marsh and 61% (120 of 198) of the tagged fish released along the Massachusetts coast were recaptured where they were released (Table 1). Across all times, of the fish recaptured where they were released (41 in GM; 120 in MA), 59% (24 of 41) were recaptured in the Great Marsh and 66% (79 of 120) were recaptured in Massachusetts 2–7 years after

**Table 1**

Recaptures of adult striped bass (*Morone saxatilis*) tagged and released in the Great Marsh region (GM), and for all Massachusetts estuaries combined (MA). Data are shown for striped bass recaptured within the first summer of release (≤104 days and before 21 September), after the first summer (2–7 years after tagging), and at all times. Detailed recapture locations are divided into two spatial scales; Great Marsh (GM) and Massachusetts (MA). For example, row 1 shows that 22 fish were recaptured in the Great Marsh in the first summer and of those 17 were released there. Recapture data are shown as numbers (*n*) and percentages (%). Numbers for recapture across scales are not exclusive and therefore columns do not sum to total recaptures. Also shown are fish tagged and released in MA in summer and recaptured in other New England states in the first summer. ME=Maine, NH= New Hampshire, RI = Rhode Island, CT= Connecticut.

| | | Recaptures | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total | GM | | MA | | ME | NH | RI | CT |
| Release location | (*n*) | (*n*) | (%) | (*n*) | (%) | (*n*) | (*n*) | (*n*) | (*n*) |
| First year | | | | | | | | | |
| GM | 22 | 17 | 77 | 22 | 100 | 0 | 0 | 0 | 0 |
| MA | 41 | | | 41 | 100 | 0 | 0 | 0 | 0 |
| 2–7 years after tagging | | | | | | | | | |
| GM | | 24 | | | | | | | |
| MA | | | | 79 | | | | | |
| Total recaptures | | | | | | | | | |
| GM | 108 | 41 | 38 | | | | | | |
| MA | 198 | | | 120 | 61 | | | | |

**Table 2**

Observed proportion of first summer recaptures of striped bass (*Morone saxatilis*) in release location from field data and weekly probabilities of model fish remaining where tagged if striped bass movements were random. Observed proportions correspond to data in Table 1. For weekly probabilities of remaining, shown are two different model scenarios (RM-1 and RM-2). Data are shown for two regions of release and recapture: the Great Marsh (GM) and all Massachusetts estuaries combined (MA). $P_e$ is the proportion that would need to remain in the recapture location each week to obtain the observed proportion of recaptures over 10 weeks.

| | | GM-weekly probability of remaining where tagged | | | MA-weekly probability of remaining where tagged |
|---|---|---|---|---|---|
| Release location | Observed recaptures (Proportion) | RM-1 $p_e$ | RM-2 $p_e$ | Observed recaptures (Proportion) | RM-2 $p_e$ |
| GM | 0.77 | 0.95 | 0.75 | 1.00 | 1.00 |
| MA | | | | 1.00 | 1.00 |

tagging (Table 1). Thus, in subsequent migration cycles, many fish were recaptured in the location where they had been tagged.

Recapture patterns were not caused by effort alone. Striped bass recaptures in both Massachusetts and the Great Marsh in the first summer after tagging were highest in July and August in 1999 and 2000 (Fig. 5, white bars), the same months when recreational fishing effort was concentrated (Fig. 5, black bars). However, when scaled by kilometer of coastline, the fishing effort for striped bass in summer was as high in Connecticut and Rhode Island as in Massachusetts and higher in New Hampshire (Fig. 6). Nevertheless, in spite of heavy effort across the entire New England coast, no striped bass tagged in Massachusetts during the summer was recaptured in any of the other four New England states in the first summer (Spearman correlation $P$=0.55).

## Discussion

Our study provides the first evidence that smaller striped bass make repeated, seasonal, long distance movements between natal and non-natal estuaries. The striped bass we tagged in Massachusetts in the summer were caught in late fall, winter, or early spring, south of the location where they had been released. If striped bass that were tagged in Massachusetts in summer were spawning or

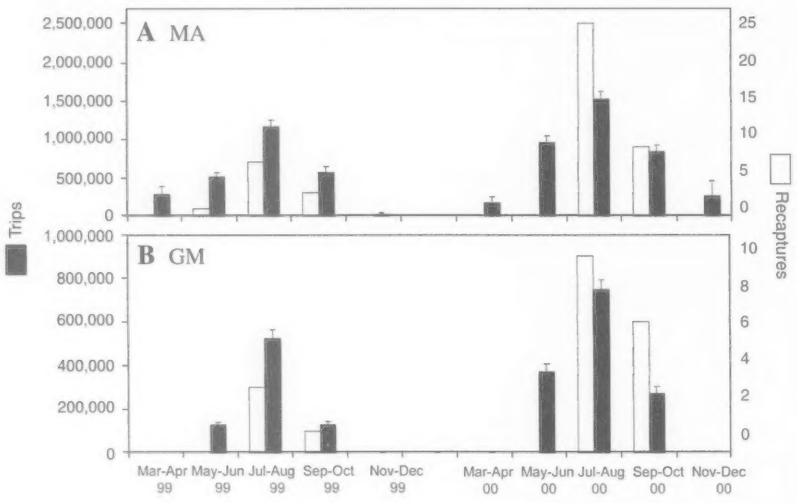


**Figure 5**

Number of first summer recaptures (white bars) and fishing effort (black bars) by date in (**A**) Massachusetts (MA), and (**B**) the Great Marsh (GM) for striped bass (*Morone saxatilis*) released there. Numbers of recaptures correspond to those in Table 1. Effort is shown in number of fishing trips for striped bass (mean ±1 SD).

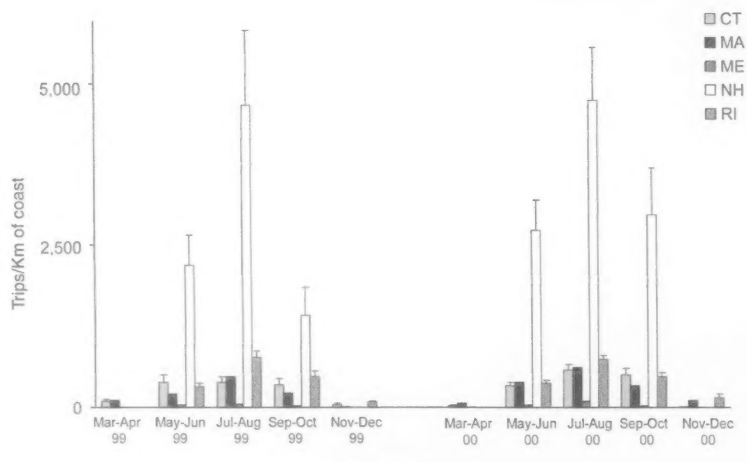


**Figure 6**

Number of fishing trips per state (±1 standard deviation), divided by the approximate km of coastline. Fish were captured by angling. Tagging occurred in 1999 and 2000. The first summer was defined as <104 days since tagging and before 21 September.

overwintering there, they would have no reason to go south towards the traditional overwintering or spawning grounds in the late fall. In addition, 33 of 46 striped bass implanted with acoustic tags in the Great Marsh, MA, in 2006 were detected by acoustic receivers in Delaware Bay or Long Island Sound (474–939 km one-way migration) in the winter, 2006–07 (Mather, unpubl. data). The U.S. Atlantic coast striped bass stocks that spawn in Chesapeake Bay, Delaware River, and the Hudson River have been observed previously off New England (Berg-

gren and Lieberman, 1978), but these coastal migrants were larger (>450 mm; Dorazio et al., 1994). We provide evidence that small adult striped bass, captured in Massachusetts during the summer, were also part of the coastal migratory stocks.

These recaptures of migratory fish at the location where they were tagged were much higher than our model predicted if movements were random. In this study, during the first summer after tagging, many striped bass were recaptured in a relatively small, local area (<20 $km^2$). Striped bass recaptured in the Great Marsh (77%) used only 0.73% of the New England coastline available for feeding by migratory striped bass. Striped bass recaptured along the Massachusetts coast (100%) used only 25.1% of the New England coastline. Furthermore, no fish tagged and released in Massachusetts in summer was recaptured in other New England states in summer, in spite of high fishing effort.

These results indicate that some highly mobile striped bass that have traveled hundreds of kilometers to feed, cease their mobile lifestyle, and remain in relatively local areas (i.e., the Great Marsh or the Massachusetts coast) for a prolonged period in summer. Summer is an important period for food acquisition, and understanding why striped bass may choose one feeding area over another is critical to managing a fish that uses multiple habitats separated by hundreds of kilometers. Migration patterns of striped bass may be linked to different conditions across habitats. High temperatures, low oxygen, and inadequate prey may deter striped bass from remaining in specific habitats (Coutant and Benson, 1990; Hartman and Brandt, 1995). In addition, growth can be higher in some locations than in others (Welsh et al., 2003). As such, migration may have evolved to move striped bass away from poor conditions (e.g., high temperature, low oxygen, or poor prey conditions) or towards better foraging conditions (e.g., seasonally abundant prey, moderate water temperatures). Prey resources may be available during a longer period of physiologically suitable conditions in northern estuaries than in southern estuaries. This feature could facilitate prolonged use of feeding habitats in northern estuaries in summer.

The degree of site fidelity observed was surprisingly high given that this highly mobile fish migrates hundreds of kilometers annually, encounters tens of estuaries, and has the potential for multidirectional movement. Site fidelity has been documented in freshwater (Buzby and Deegan, 2000; McCairns and Fox, 2004), marine (Thorrold et al., 2001; Szedlmayer and Schroepfer, 2005), and anadromous fish (Stewart et al., 2004; Minakawa and Kraft, 2005). However, many of these examples of site fidelity are within physically constrained riverine systems. Some site fidelity has been observed for striped bass in freshwater (Jackson and Hightower, 2001), but it has only recently been examined for coastal migrants (Wingate and Secor, 2007; this study).

Home range, or the habitats used over a period of time (day, season, year), can be a useful way of thinking about a species distribution and its relationship to environmental conditions. The size of a home range can vary with sex, season, availability of resources, body size, feeding strategy, and group size (Baker, 1978). Although home range can be measured for any animal, the concept of home range may make little sense for animals that roam widely. For migratory fish that feed throughout their entire range or that use a large ocean feeding area, the concept of a feeding home range may have limited utility. However, a feeding home range provides a useful conceptual framework for understanding movements of fish like striped bass that may migrate between discrete spawning and feeding areas. This discrete feeding home range of individual fish can also have implications for fisheries management. Migratory fish that stay in a specific area for a prolonged time may adopt different feeding strategies based on previous experience in the estuary, and these feeding strategies may result in spatially explicit patterns of growth. In addition, local fishing pressure may influence feeding groups such that migratory fish that stay in a local area for a prolonged period may be caught and released more frequently, resulting in higher mortality. Consequently, knowing the movements of individual migratory fish in foraging areas is crucial for appropriate management.

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# Surface mucous as a source of genomic DNA from Atlantic billfishes (Istiophoridae) and swordfish (Xiphiidae)

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**Abstract**—Procedures for sampling genomic DNA from live billfishes involve manual restraint and tissue excision that can be difficult to carry out and may produce stresses that affect fish survival. We examined the collection of surface mucous as a less invasive alternative method for sourcing genomic DNA by comparing it to autologous muscle tissue samples from Atlantic blue marlin (*Makaira nigricans*), white marlin (*Tetrapturus albidus*), sailfish (*Istiophorus platypterus*), and swordfish (*Xiphias gladius*). Purified DNA from mucous was comparable to muscle and was suitable for conventional polymerase chain reaction, random amplified polymorphic DNA analysis, and mitochondrial and nuclear locus sequencing. The nondestructive and less invasive characteristics of surface mucous collection may promote increased survival of released specimens and may be advantageous for other marine fish genetic studies, particularly those involving large live specimens destined for release.

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Understanding genetic variation and identifying evolutionary lineages are important considerations for conservation management of large pelagic fishes (Palumbi, 1994; Ward, 2000). Some methods used for genetic sampling of billfishes (families Istiophoridae and Xiphiidae) require excision of muscle, skin, or fin tissue, and excision requires manual restraint or killing of the animal. Some nondestructive sampling methods, such as fin and scale collection, exist that avoid the need to kill the fish (Yue and Orban, 2001; Wasko et al., 2003; Hoolihan et al., 2004). Tissue excision from very large live specimens is problematic because of personal safety concerns for handlers, and injuries to fish that may reduce survival. Most recreational billfish caught in the United States are released (Prince et al., 2007), whereas possession of billfish by U.S. pelagic longline vessels and sales of Atlantic billfishes have been prohibited since 1988. This regulation eliminates opportunities to obtain DNA samples from landed specimens, warranting a need for alternative methods. To sample genomic DNA with nondestructive techniques and with minimal handling would promote the survival of released individuals and increase the opportunities to conduct genetic studies.

One alternate potential source of genomic DNA is the epidermal cells found in billfish and swordfish surface mucous. Successful extractions of sufficient quantities of genomic DNA from surface mucous of freshwater fishes have been reported for *Salmo trutta fario* (brown trout), *Esox lucius* (northern pike) (Livia et al., 2006) and *Scleropages formosus* (Asian arowana) (Chansue, 2006), providing an experimental model for large pelagic species.

The present study compares DNA extractions from surface mucous and autologous skeletal muscle tissue from billfishes and swordfish to determine the suitability of DNA extracted from surface mucous for random amplified polymorphic DNA (RAPD) and DNA sequencing analyses.

## Materials and methods

Surface mucous and autologous skeletal muscle samples were collected

from three individuals from each of the following species: *Makaira nigricans* (blue marlin), *Tetrapturus albidus* (white marlin), *Istiophorus platypterus* (sailfish), and *Xiphias gladius* (swordfish). We compared purified DNA extracted from mucous and muscle using RAPD, mitochondrial DNA (mtDNA) sequencing, and nuclear DNA sequencing analyses.

### Sample preparation and DNA purification

Surface mucous samples were collected with a polyurethane sponge as described by Schultz et al. (2006) and immediately processed, or stored at −80°C for later extraction. Each sponge was cut into small pieces, mixed with 1500 μL of phosphate buffered saline, and compressed repeatedly to remove mucous. All fluids were centrifuged through a single QIAamp® (Qiagen Inc., Valencia, CA) spin column. DNA purification was carried out by using Qiagen® buccal swab spin protocol with the following modifications: 1) a final concentration of 1 millimolar (mM) ethylenediaminetetraacetic acid (EDTA) was added to the phosphate-buffered saline extraction buffer; 2) the proteinase K treatment step was eliminated; and 3) an RNase mixture of A and T1 enzymes was used to degrade RNA after the final DNA purification step (Ambion Inc., Foster City, CA). The RNase cocktail enzyme mix was necessary because the Qiagen® spin columns copurify RNA and DNA in parallel when both are present in a sample. Cold ethanol precipitation and inclusion of EDTA in buffers were used to reduce nuclease degradation (Dessauer et al., 1996; Wasko et al., 2003).

For muscle tissue, 25 mg were macerated in 180 μL Buffer ATL (Qiagen Inc., Valencia, CA), and incubated overnight in a 56°C water bath in the presence of 20 μL proteinase K. Purification followed the QIAamp® manufacturer's protocol for tissue. Aqueous samples of nucleic acid (1.5 μL) from mucous and muscle extractions were measured for purity with a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific,Wilmington, DE) and showed a range of 1.7 to 2.0 for the DNA and RNA absorbance ratios (260 nm:280 nm).

### RAPD analysis

Three different 10-mer oligonucleotide primers (Table 1) of arbitrary sequence (IDT Integrated DNA Technologies, Coralville, IA) were tested against surface mucous DNA and autologous muscle DNA from three individuals of *M. nigricans*, *T. albidus*, *I. platypterus*, and *X. gladius*. PCR reactions for RAPD analysis were performed in a total volume of 25 μL containing 3 μL extracted genomic DNA, 2.5 μL of 10 mM 10-mer primer, 2.5 μL of 2.5 mM deoxynucleotide triphosphate (dNTPs), 0.75 μL of 25 mM $MgCl_2$, 0.20 μL *Taq* DNA polymerase, 2.5 μL 10× buffer, 5 μL 5M betaine (N, N, N-trimethylglycine), 2 μL HotStart-IT® binding protein (USB Corp., Cleveland, OH), and 6.55 μL distilled water. Pre-PCR incubation with the HotStart-IT™ binding protein was executed at 25°C for four hours to prevent mispriming and primer dimerization during amplification (Chou et al., 1992). RAPD PCR was performed in an Eppendorf Mastercycler (Westbury, NY) starting with initial heating for 5 minutes at 94°C, followed by 34 cycles at 94°C for 5 minutes, 42°C for 30 seconds, 72°C for 90 seconds, and a final extension of 72°C for 10 minutes. A negative control (no genomic DNA) was included in each PCR set to verify no reagent contamination. The PCR products were verified by electrophoresing 5 μL in 1.2% agarose gel (ISC BioExpress, Kayville, UT) and TAE buffer (pH 8.5) for 60 min at 100 V (60 mA), stained with ethidium bromide, and visualized through a UV transilluminator.

**Table 1**

Nucleotide composition of three 10-mer primers used for random amplified polymorphic DNA (RAPD) comparison of DNA extracted from billfish surface mucous and muscle tissue, and percent content of guanine and cytosine (% GC).

| Primer no. | Nucleotide composition | % GC |
|---|---|---|
| 1 | 5′-GTTGCGGGCT-3′ | 70 |
| 2 | 5′-CAGCCCGGGT-3′ | 80 |
| 3 | 5′-AGGCCACCGC-3′ | 80 |

### Sequencing analysis

Mitochondrial and single-copy nuclear loci were sequenced from three individuals from each of the following species: *M. nigricans*, *T. albidus*, and *I. platypterus*. Insufficient samples of mucous and muscle were available for sequencing *X. gladius*. Mitochondrial NADH dehydrogenase subunit4 (ND4) was amplified by PCR with the primer pair 61F and 1837R and cycling parameters outlined in Shivji et al. (2006). All amplifications of the anonymous single-copy nuclear locus WM13 were performed with the primer pair WM13-F and WM13-R developed by Buonaccorsi et al. (1999). Nuclear PCR reactions were performed in a total reaction volume of 50 μL containing 1 μL of extracted genomic DNA, 10 pmol/μL of each primer, 40 μM dNTPs, 10× PCR buffer, and 1 unit of HotStar *Taq*™ DNA Polymerase (Qiagen Inc., Valencia, CA). The PCR thermal profile consisted of an initial heating at 95°C for 15 minutes to activate the DNA polymerase, followed by 35–40 cycles at 94°C for 1 minute, 1 minute at 50°C, and 1 minute at 72°C, with a 5-minute final extension step at 72°C. Both nuclear and mitochondrial amplifications were performed in a MJ Research PTC-200 thermal cycler (Waltham, MA). A negative control (no genomic DNA) was included in each PCR set to verify that there was no reagent contamination.

All amplified products were purified by using the QIAquick® PCR purification kit (Qiagen Inc., Valencia, CA) and sequenced with an Applied Biosystems 3130

genetic analyzer (Foster City, CA). Forward and reverse sequences were assembled and edited with GeneDoc 2.6.002 (http://www.psc.edu/biomed/genedoc, accessed 1 June 2008). To control for cross-contamination with other billfish or possible parasites or microbes, we compared the mitochondrial and nuclear sequences derived from surface mucous to sequences obtained from autologous muscle tissue. Because autologous muscle tissue was not available for the *T. albidus* sequencing analyses, the mucous sample sequences were compared to homologous locus sequences obtained from reference *T. albidus* tissues available in our laboratory.

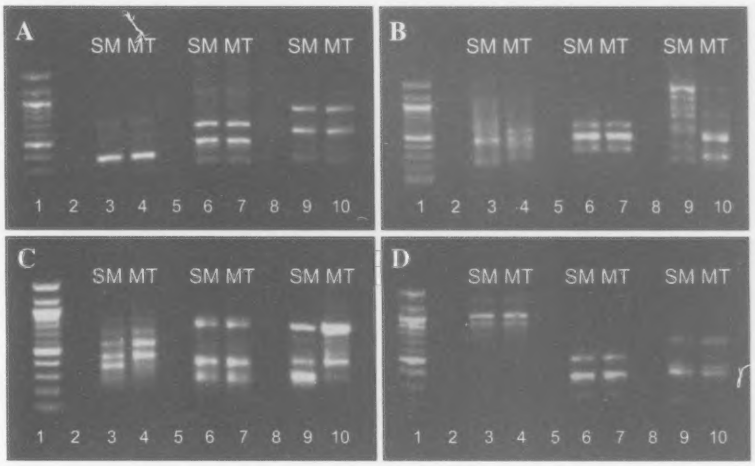


**Figure 1**

Representative random amplified polymorphic DNA (RAPD) banding patterns from four species: **A**) *Makaira nigricans* (blue marlin), **B**) *Tetrapturus albidus* (white marlin), **C**) *Istiophorus platypterus* (sailfish), and **D**) *Xiphias gladius* (swordfish) for three 10-mer primers (Table 1) tested on each fish for the template DNA from surface mucus (SM), and autologous muscle tissue (MT). Lane contents are as follows: 1, 100-bp ladder; 2, 5, and 8, negative controls (no DNA); 3 and 4, primer no. 1; 6 and 7, primer no. 2; and, 9 and 10, primer no. 3.

## Results and conclusions

### RAPD analysis

Comparable RAPD amplicons were derived from surface mucous and autologous muscle tissue of *M. nigricans*, *T. albidus*, *I. platypterus*, and *X. gladius* for each of the three 10-mer primers tested. Representative RAPD banding patterns are illustrated in Figure 1. The negative control reactions (minus template DNA) observed with each primer confirmed that the amplified genomic DNA from both sources was not an artifact of the primer concentration. Results indicated that sufficient quantities of genomic DNA are available in surface mucous from all the species tested.

### Sequencing analysis

For the three species screened, *M. nigricans*, *T. albidus*, and *I. platypterus*, the quality of nuclear and mitochondrial PCR amplifications were comparable. For both the nuclear WM13 and the mt ND4 locus, sequences derived from a single individual from both sources of genomic DNA (mucous and autologous muscle tissue) were found to be identical when compared with respect to nucleotide base composition (5′–3′ and 3′–5′ directions), thereby demonstrating that the genomic DNA derived from surface mucous was not due to cross-contamination from other billfish or microbes, and in fact originated from the fish sampled. Nuclear and mitochondrial sequences derived from both sources of genomic DNA are available from GenBank under the following accession numbers: *T. albidus*: ND4 (997bp): FJ809995-FJ809997; WM13 (279bp): FJ809988; *M. nigricans*: ND4 (966bp): FJ809991, FJ809992, and FJ809994; WM13 (279bp): FJ809986, FJ809987, and FJ809989; *I. platypterus*: ND4 (1009bp): FJ809990 and FJ809993; WM13 (277bp): FJ809984 and FJ809985.

This study has shown that surface mucous contains sufficient quantities of genomic DNA to carry out RAPD analyses of istiophorid billfishes and swordfish, as well as sequencing applications of istiophorid billfishes. These extractions compare favorably to genomic DNA extractions from surface mucous reported for freshwater species *S. formosus* (Chansue, 2006), *E. lucius*, and *S. trutta fario* (Livia et al., 2006).

The slight differences between some of the mucous and muscle RAPD amplification profiles (Fig. 1) may be a result of contaminant DNA in the mucous (e.g., bacteria, microalgae), or other artifact variations known to occur in RAPD studies (Ellsworth et al., 1993). We found that the pre-PCR incubation of the template DNA master mix with HotStart-IT™ binding protein was crucial for preventing mispriming and primer dimerization that produced these artifact bands (Chou et al., 1992). In addition, the inclusion of betaine (N, N, N-trimethylglycine) improved band visibility by eliminating the smearing attributed to the formation of secondary structure, which is caused by G-C rich regions (Henke et al., 1997). Importantly, for surface mucous RAPD analysis, the RNAase treatment of nucleic acid was a necessary step, whereas the inclusion of proteinase K was not.

The nondestructive and less invasive procedures associated with sampling surface mucous offers many advantages when compared to fin clip and muscle tissue collection, particularly when very large fishes destined for live release are involved. Although the collection of surface mucous with sponges was adequate for the present study, commercially available products such as FTA® cards (Whatman Inc., Florham Park, NJ) may offer advantages. For example, Livia et al. (2006) reported that FTA® cards were a fast and reliable method of collecting, storing, and extracting genomic DNA from *E. lucius* and *S. trutta fario*. FTA® cards can be stored dry at room temperature, thus eliminating the need for laboratory freezers or special shipping considerations. We tested FTA® cards on a limited number of *I. platypterus* and *T. albidus* surface mucous samples (data not shown). Preliminary results were successful for PCR amplification, and further analyses are planned.

We report the first use of surface mucous from marine fishes as an alternative method of DNA sampling. As such, the method offers advantages that warrant consideration when planning genetic studies on other marine species, particularly those where live-release is desirable.

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# Assessment of habitat quality for juvenile California halibut (*Paralichthys californicus*) in a seasonally arid estuary

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**Abstract**—We evaluated habitat quality for juvenile California halibut (*Paralichthys californicus*) in a Pacific Coast estuary lacking in strong salinity gradients by examining density, recent otolith growth rates, and gut fullness levels of wild-caught and caged juveniles for one year. Juveniles <200 mm standard length were caught consistently in the inner, central, and outer sections of the estuary. The density of juveniles was two times higher in the inner estuary during most of the year, consistent with active habitat selection by part of the population. A generalized linear model indicated temperature, sampling time, and the interaction between salinity and temperature were significantly related to density. However, the model explained only 21% of the variance. Gut fullness levels of wild-caught juveniles were highest during the summer, but recent otolith growth rates were not related to temperature. The proportion of individuals feeding successfully indicated that seasonal differences in food availability are more important than spatial variation in prey abundance in driving feeding success. Feeding success of caged fishes was limited, precluding the use of growth rates as indicators of local habitat quality. However, marginal increment widths were reliable indicators of somatic growth at low growth rates over two-week periods. The relatively high growth rates and abundance of small wild-caught juveniles found throughout the estuary indicates that the entire estuary system has the potential for serving as nursery habitat.

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California halibut (*Paralichthys californicus*) is a species of commercial and recreational importance that is found in coastal waters from Washington, U.S.A., to Baja California Sur, Mexico (Sweatnam et al., 2007). Spawning occurs along coastal areas and planktonic larvae inhabit the continental shelf for about a month before settling in shallow protected areas along the coast or in the outer reaches of protected embayments (Allen and Herbinson, 1990; Moser and Watson, 1990). Juveniles make facultative use of protected embayments such as coastal lagoons, bays, and estuaries (Horn and Allen, 1985; Allen, 1988; Kramer, 1990; Fodrie and Mendoza, 2006). Recent studies indicate that both protected embayments and coastal habitats contribute to the production of adults, but that juvenile densities are substantially higher within embayments (Forrester and Swearer 2002; Fodrie and Mendoza, 2006; Fodrie and Levin, 2008). However, there are no studies where juvenile habitat quality has been assessed for California halibut on the intra-embayment level.

Juvenile flatfish occupying specific habitats or areas within embayments may exhibit considerable variability in abundance, growth, and mortality rates (Allen and Baltz, 1997; Gilliers et al., 2006). In addition, specific regions within embayments may contribute disproportionaly to the production of recruits to the adult population (Beck et al., 2001). Sogard (1992) reasoned that if rapid growth during the juvenile stage offers an advantage in terms of increased survival, and if growth rates vary as a function of habitat quality, individuals will select habitats that offer the maximum growth potential, although biological interactions such as predation can influence habitat choice. Within this context, high-quality nursery habitats within embayments are those in which growth and survival rates are higher than the corresponding rates in coastal habitats (Gilliers et al., 2006).

In estuarine systems strongly influenced by freshwater inflow ("classical" estuaries), salinity exhibits strong spatial gradients from the mouth to the head of the estuary. Gradients in salinity have been related to the distribution of some species of flatfish (e.g., Allen and Baltz, 1997; Able et al., 2005). In contrast, estuaries in seasonally arid regions, such as the Pacific coast of Baja California and southern California, tend to receive freshwater inflow only during the rainy winter season or summer monsoon. These "Mediterranean-type" estuary systems are increasingly hypersaline with increasing distance from the river mouth during a large part of the year, and in the inner reaches of the estuaries the water exhibits high residence times, which influence nu-

trient availability and production (Largier et al., 1997). Hence, spatial patterns of habitat use and quality for juvenile flatfish in classical and "Mediterranean-type" estuaries may differ substantially.

Use of habitat by juvenile flatfish has been related mostly to temperature, salinity, dissolved oxygen, substrate type, and depth (Gibson, 1994; Able et al., 2005). The first three variables directly influence metabolic processes, and hence abundance and growth (Gibson, 1997). Substrate type can be used as a proxy for food availability, and is related to success in predator avoidance (Gibson, 1994; Amezcua and Nash, 2001). Differences in distribution as a function of depth may be due to habitat partitioning among life stages (Kramer, 1990; Gibson, 1997). Because a direct assessment of juvenile habitat quality is difficult, growth and density have served as proxies. These measures integrate the effect of biological factors and environmental conditions (Necaise et al., 2005; Gilliers et al., 2006). Specifically, estimates of recent otolith growth rates evaluated through the measurement of increment widths can be used as an integrative indicator of habitat quality and the suitability of environmental conditions over short time scales (Le Pape et al., 2003; Gilliers et al., 2006). The fine-scale temporal and spatial variation of growth rates can thus serve as indicators of habitat quality.

The use of otolith marginal increment widths as indicators of habitat quality relies on two premises. First, there must be a high correlation between somatic and otolith growth rates (Campana and Jones, 1992). This has been shown to be the case for juvenile California halibut (Kicklighter, 1990; Kramer, 1991). Secondly, differences in growth rates should reflect the quality of the habitat in which fish were captured (Sogard, 1992; Gilliers et al., 2006). The second premise can be rendered invalid if there is substantial movement to or from areas with different environmental conditions within the time interval used to evaluate growth. Haaker (1975) found little movement of tagged juvenile California halibut within Anaheim Bay in southern California. Tagging studies of age-0 winter flounder (*Pseudopleuronectes americanus*) and plaice (*Pleuronectes platessa*) have also indicated limited displacement on the scale of a few hundred meters (Saucerman and Deegan, 1991; Burrows et al., 2004). However, Fodrie and Herzka (2008) used otolith microchemistry to reconstruct movement patterns of juvenile California halibut within an arid estuary and found that 8 out of 14 (57%) individuals moved among sections of the estuary over a two-month period. Likewise, Herzka et al. (2009) examined length-frequency distributions and recapture locations of tagged individuals and found simultaneous evidence of estuarine emigration and residency. If juveniles move substantially within an estuary, the implicit assumption that an individual has remained in the vicinity of its capture location for the time period over which growth is evaluated may be violated. Caging experiments guarantee that an individual has remained at a given location under measurable environmental conditions (Sogard, 1992). However, confinement has the potential to influence natural growth rates (Guindon and Miller, 1995). Caging experiments and sampling of natural populations are thus complimentary approaches for using growth rates as proxies for habitat quality.

We evaluated nursery habitat quality for juvenile California halibut (*Paralichthys californicus*) in a seasonally arid estuary, Punta Banda Estuary in Baja California, Mexico, based on recent otolith growth rates and an index of feeding success in relation to feeding levels. To determine if specific sections of the estuary serve as preferred juvenile habitat, we assessed spatial and temporal variability in density and evaluated recent otolith growth rates in relation to environmental conditions. In addition, we tested the hypothesis that juvenile density is higher in areas that favor higher growth rates. We simultaneously performed caging experiments and sampled natural populations to assess growth rates in relation to environmental conditions.

## Materials and methods

### Study area

Punta Banda Estuary is a medium-size (11-km$^2$ at high tide, 5-km$^2$ at low tide) protected embayment located within the Southern California Bight. It is located 100 km south of the US-Mexico border on the Pacific side of Baja California, Mexico (Fig. 1). The estuary lies along the southeastern margin of Todos Santos Bay (31°42′–31°47′N lat and 116°37′–116°39′W long), a semiprotected coastal system. The L-shaped estuary is connected to the bay at its northern end through a 125-m inlet (Ortiz et al., 2003). The fastest current velocities are found within the main channel at the mouth (~1 m/s), where depths are 10–12 m (Pritchard et al., 1978). A channel runs along the main axis of the estuary and is flanked by shallow submerged or exposed flats at low tide. The depth of the main channel in the central and inner sections of the estuary is shallow (<3 m) compared to the outer section (approximately 8 m, see Ortiz et al., 2003 for a detailed bathymetry). Along the shorter axis, the main channel splits into different arms and the average depth is <1 m in relation to mean low water. Patches of eelgrass (*Zostera marina*) are found in the central estuaries and there are tidal marshes along most of the banks. The bottom is sandy towards the outer reaches of the system and becomes increasingly silty toward the head (Ortiz et al., 2003).

Because the estuary is located in a seasonally arid region, the estuary behaves mostly as a negative estuary, in that temperature and salinity increase from the mouth to the head, particularly during the warmer months (Álvarez-Borrego and Álvarez-Borrego, 1982). Temperature and salinity also exhibit variations associated with the semidiurnal tidal cycle because of the exchange of water with Todos Santos Bay. Maximum tidal range during spring tides is about 1.7 m.

### Experimental design

Density, recent growth rates, and environmental parameters (temperature, salinity, dissolved oxygen and gut fullness levels as a proxy for food availability) were measured approximately every other month between October 2004 and October 2005. To evaluate growth and abundance over a gradient of environmental conditions, the inner, central and outer reaches of the estuary were chosen as sampling and caging locations (Fig. 1). Six 28-day caging experiments were performed throughout the year to evaluate recent otolith growth, somatic growth, and gut fullness levels (Table 1). Density of natural populations was evaluated at the beginning and end of each caging experiment. Juveniles collected at the beginning of each caging period were used for caging experiments, whereas those captured at the end were used for measuring otolith growth rates and evaluating gut fullness levels of natural populations. To minimize the range of sizes evaluated for growth and gut fullness levels and allow for direct comparison of otolith growth rates from natural populations and caged individuals, we limited the sizes of fish analyzed to between 50 and 160 mm standard length (SL).

### Density and environmental variables

On each sampling date, five 10-minute tows were conducted in each section of the estuary with a 7.6-m wide otter trawl (headrope length 9.5 m, 2.0- and 0.5-cm mesh in the body and codend, respectively). The sampling gear was not efficient at catching fish <40 mm SL. For each section and sampling date, we sampled at a range of depths and areas to obtain a representative index of density. Sampling was mostly limited to the mid-to-high tidal range of spring tides because we needed to gain access to the cages during low tides. All tows were performed with the prevailing current at a target tow speed of 3 km/hr. For each tow, initial and final coordinates, mean current speed and the distance covered by the trawl (approximately 500 m) were registered by using a hand-held GPS. Temperature, salinity and dissolved oxygen (DO) were measured at the end of each tow with an YSI 85 dissolved oxygen and conductivity meter (Yellow Springs Intruments, Concord, CA). Because previous studies have reported a relationship between depth and abundance of juvenile California halibut in shallow estuarine systems (Kramer, 1990; Fodrie and Mendoza, 2006), a consistent depth was maintained during each tow. The depth of each tow was monitored and recorded with an onboard fish finder. To obtain a density estimate representative of each section of the estuary, tows were performed at a variety of depths and locations.

After capture, California halibut were immediately measured for standard length. Length-frequency distributions were constructed for each sampling period and section of the estuary by 10-mm size classes. Density on an area basis was calculated by assuming a 7.6-m trawl width and standardizing the tow to 1000 $m^2$ (reported as fish/1000 $m^2$). Estuarine emigration has been reported to occur during summer at lengths ranging from 140 to 200 mm (Haaker, 1975; Kramer, 1990; Hammann and Ramírez-González, 1990). Hence, mean density (±standard error [SE]) was calculated for fish ≤200 and >200 mm SL for each section of the estuary and sampling time.

Given that our primary interest was young juveniles, the densities of fish ≤200 mm SL were analyzed by two-way analysis of variance (ANOVA) with time and section of the estuary as fixed factors. Normality and homogeneity of variance were evaluated by using the Kolmogorov-Smirnov and Levene's tests, respectively. The data were log ($x$+1) transformed before we conducted the ANOVA to comply with the assumption of normality. A Tukey HSD test for unequal sample sizes was used to identify homogenous groups. The size-frequency

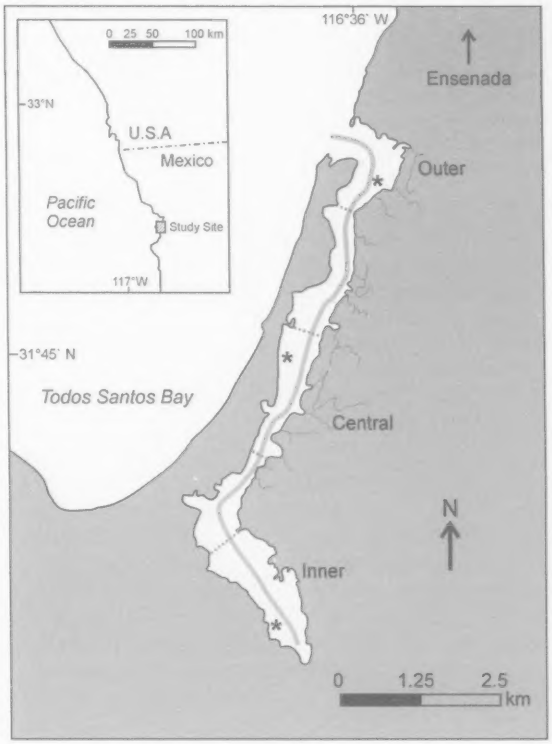


**Figure 1**

Map of Punta Banda Estuary, Baja California, Mexico where density and growth rates of California halibut (*Paralichthys californicus*) were assessed between October 2004 and October 2005. Black asterisks indicate locations where experiments were performed. Sampling for natural abundance and growth estimates was conducted in the outer, central, and inner sections of the estuary; the areas between these sections were not sampled. The gray solid line indicates the channel that runs along the main axis of the estuary.

**Table 1**

Starting date, size range, and number of fish recovered in cages in the outer, central, and inner reaches of Punta Banda Estuary. Cages were recovered after 14 or 28 days; each row indicates a single cage ($n$=6 juveniles). Asterisks indicate locations in which cages were not recovered. No data (n. d.); cages were not seeded because of low capture rates.

| | | 14-day caging period | | 28-day caging period | |
|---|---|---|---|---|---|
| Starting date | Section | Size range mm SL | Number of fish recovered (% mortality) | Size range mm SL | Number of fish recovered (% mortality) |
| 28 Oct 2004 | Outer | 89–158 | * | 72–130 | * |
| | Outer | 70–102 | * | 69–85 | * |
| | Central | 96–159 | 6 (0) | 55–103 | 6 (0) |
| | Central | 79–135 | 6 (0) | 67–146 | 4 (33) |
| | Inner | 78–146 | 6 (0) | 75–149 | * |
| | Inner | 75–150 | 4 (33) | 67–148 | * |
| 12 Jan 2005 | Outer | 96–116 | 4 (33) | 80–145 | * |
| | Outer | 84–104 | 0 (100) | 82–143 | * |
| | Central | 88–115 | 5 (17) | 90–122 | 6 (0) |
| | Central | 90–113 | 6 (0) | 62–146 | 5 (17) |
| | Inner | 113–149 | 4 (33) | 120–140 | 4 (33) |
| | Inner | 95–112 | 4 (33) | 85–119 | 2 (67) |
| 28 Mar 2005 | Outer | 61–125 | 1 (83) | n.d. | — |
| | Outer | 55–140 | 5 (17) | n.d. | — |
| 10 Mar 2005 | Central | 62–138 | 4 (33) | 54–150 | 4 (33) |
| | Central | 52–136 | 5 (17) | n.d. | — |
| | Inner | 80–150 | 6 (0) | 118–156 | 6 (0) |
| | Inner | 80–144 | 5 (17) | 102–160 | 6 (0) |
| 04 May 2005 | Outer | 114–140 | 4 (33) | 63–132 | * |
| | Outer | 65–140 | 2 (67) | 64–155 | * |
| | Central | 64–165 | 5 (17) | 69–157 | 5 (17) |
| | Central | 63–152 | 6 (0) | n.d. | — |
| | Inner | 68–150 | 4 (33) | 57–160 | 4 (33) |
| | Inner | 56–139 | 4 (33) | 56–146 | 4 (33) |
| 16 Jun 2005 | Outer | 88–146 | 3 (50) | 89–159 | * |
| | Outer | n.d. | — | 106–133 | * |
| | Central | 91–157 | 6 (0) | 101–161 | 3 (50) |
| | Central | 72–99 | 3 (50) | 69–73 | * |
| | Inner | 65–170 | 4(33) | 72–150 | 5 (17) |
| | Inner | n.d. | — | 63–172 | 4 (33) |
| 06 Sep 2005 | Outer | 75–122 | 4(33) | 71–144 | * |
| | Outer | 73–141 | 6 (0) | 71–143 | 6 (0) |
| | Central | 53–146 | 4(33) | 50–151 | 4 (33) |
| | Central | n.d. | — | 110–129 | 4 (33) |
| | Inner | 91–167 | 5 (17) | 147 | 1 (83) |
| | Inner | n.d. | — | n.d. | — |

distributions of California halibut ≤200 mm SL caught in different sections of the estuary were compared by using the nonparametric Kruskall-Wallis $H$ test.

The relationship between density of fish ≤200 mm SL, environmental conditions, section of the estuary and sampling time was evaluated by using a general linear model (GLM). Densities were log($x$+1) transformed to achieve normality. Temperature, salinity, DO, and depth were were included as continuous predictor variables. Section of the estuary and sampling time were included as categorical variables. Data corresponding to the beginning and end of each caging experiment were pooled for analysis and considered as a single point in time; the data from August 2005 were entered as a separate time point. Because there were significant correlations between salinity and tempreature ($r$=0.487) and salinity and DO ($r$=−0.633), the model was run to include an interaction term for these variables. Model

residuals were close to normally distributed (Shapiro Wilk's statistic=0.984, $P$=0.031).

### Otolith growth rates of natural populations

Using measurements of daily increment widths as a proxy for somatic growth rate, we relied on two assumptions: 1) that growth increments in the form of ring formations in otoliths occurs daily, and 2) the increment widths of otoliths are proportional to somatic growth (i.e., there is a linear relationship between fish size and otolith size; Campana and Jones, 1992). Kramer (1991) and Kicklighter (1990) validated daily ring formation in larval and juvenile California halibut, respectively. Using caging experiments to evaluate otolith and somatic growth rates in juvenile California halibut, Kicklighter (1990) documented a strong linear relationship between SL and otolith length for juveniles ranging from 40 to 100 mm SL. Kicklighter (1990) also reported a linear relationship between mean recent otolith growth (14 days) and somatic growth ($y$=0.619$x$+0.093, $r^2$=0.55). Hence, the measurement of recent increment widths as a proxy for somatic growth rates in juvenile California halibut is justified.

Wild-caught juveniles were placed over ice in individual bags and later frozen in the laboratory. We chose five individuals from each of the following size classes for otolith analysis: 50–80, 81–120 and 121–160 mm SL ($n$=15 per date and section of the estuary). Sagittal otoliths were extracted, cleaned in a sonicator with a 10% bleach solution to remove tissue, rinsed with distilled water, dried, and mounted on slides sulcus-side-down with Krazy Glue (Industrias Kola Loka SA de CV, Mexico State, Mexico). The right or left sagittal otolith was chosen randomly. The percent difference in length between the sagittal otoliths dissected from the eyed or blind side of both right and left-eyed juveniles is small (mean ±standard deviation [SD] in relation to blind side=–1.7% ±3.0%, range –11.1% to 2.5%, $n$=20).

Polishing was necessary to reveal daily growth increments in the otolith posterior margin. Polishing cloths of several sizes (34.3, 22.1, 14.5, and 6.5 μm) were used depending on otolith size and visibility of daily increments. Otoliths were given a final polishing with a 0.3-μm aluminum powder and soaked in 5% EDTA to increase the visibility of daily growth increments.

We were interested in examining the relationship between recent growth rate and environmental conditions over a relatively short time period to minimize the possibility that individuals had moved substantially within the estuary. We measured the width of daily growth increments for the period corresponding to the 14 days before capture (corresponding to the second half of the caging experiment). Daily increments, consisting of an opaque and translucent ring, were viewed under 400× magnification. The width of each increment was measured parallel to the main growth axis of each otolith in the posterior margin (range of widths 3–15 μm; mean= 6 μm ±2 SD) by using an imagine analysis system consisting of a compound microscope and digital camera connected to a computer loaded with Image J analysis software (National Institutes of Health, Bethesda, MD). We had difficulty distinguishing the interface between the opaque and translucent rings of the outermost increments with sufficient clarity to accurately measure the daily increment widths, although we could identify daily increments. We therefore counted the 14 increments deposited before capture and measured the width of those in which the interface between the opaque and translucent rings was clear to obtain an accurate measurement. The number of increments measured in a given otolith ranged from 5 to 12 (mean=9 ±3 SD). Otoliths for which we could not measure at least five increments were discarded (about 20%). In those cases, we prepared additional otoliths from fishes of the same size class. Each increment was measured three times and its average width was used in subsequent calculations. Recent growth rates are reported in μm/day.

The width of daily increments can vary as a function of fish size. Analysis of covariance (ANCOVA) has been used in previous studies to remove size-related differences in tests for differences in otolith growth rates among groups (e.g., Phelan et al., 2000). We evaluated whether recent otolith growth was correlated with SL within the 50–160 mm SL size range that we examined. Data from fishes collected in different sections of the estuary at a given time were pooled. Sampling periods were considered separately and six correlations were performed ($n$=45 fish per sampling period). Only one correlation was positive and significant (October 2004; $P$<0.001) and a second was slightly negative and marginally significant (January 2005; $P$=0.032). In both cases, the proportion of the variance in recent otolith growth rates explained by SL was very low ($r^2$=0.26 and $r^2$=0.10, respectively). Hence, we did not find a strong dependence of recent otolith growth rates on size within our target size range. Recent otolith growth rates of natural populations were thus analyzed withg two-way ANOVA and by using time and section of the estuary as fixed factors. Data were log ($x$+1) transformed to comply with the assumption of normality. Tukey HSD tests were used to test for specific differences between means after ANOVA.

We used correlation analysis to test the hypothesis that higher growth rates coincide with higher density of juveniles ≤200 mm SL and to examine the relationship between recent otolith growth rates of natural populations and temperature. Mean temperature during the 14 days before their capture was calculated by using data from thermographs deployed during caging experiments (see below). Correlation analysis was performed only for times and sections of the estuary for which both otolith and temperature data were available.

### Somatic and otolith growth rates of caged fishes

Valle et al. (1999) examined the fine-scale distribution of juvenile California halibut in shallow (<1.1 m depth) waters of Alamitos Bay, CA, in relation to the presence or absence of eelgrass habitat. Juveniles were 2–6 times

more abundant in unvegetated habitats. Eelgrass is not found in the outer and inner sections of Punta Banda Estuary and only sparse eelgrass beds are found in the central section. We placed cages solely in unvegetated areas because eelgrass is not the preferred habitat of juvenile halibut. Cages were constructed with a PVC frame (1 m wide × 1 m long × 0.5 m high) wrapped in Vexar® low-density polyethylene netting of 0.5 × 0.5-cm mesh. Cages were closed on the bottom to prevent escapement. Similar designs have been used in other studies seeking to evaluate the growth of juvenile flatfish (Kicklighter, 1990; Sogard, 1992). Caging experiments began and ended during spring tides, allowing us access to subtidal habitat during lower low water. The day before starting each caging experiment, four cages were placed within a few meters of each other in the outer, central, and inner sections of the estuary (Fig. 1). Each cage was anchored by rebar fastened to the corners with cable ties. Temperature was measured continuously at each caging location by using thermographs placed in the vicinity of each caging location (HOBO Water Temperature Data Loggers model 856097, Onset Computer Corporation, Pocasset, MA). These thermographs were programmed to record every 30 minutes.

To supply the cages, individuals within the target size range (50–160 mm SL) were placed in ice chests filled with aerated seawater immediately after trawling. Seawater was exchanged frequently and mortality was minimal. Cages were seeded with juveniles caught in the same section of the estuary to avoid subjecting them to substantial changes in the environmental conditions to which they had been exposed.

The target number of fish to be placed within each cage was six. Other flatfish studies have used similar fish densities (Kicklighter, 1990). The number of fish introduced into each cage was held constant throughout the caging experiments. If we did not catch enough fish of the targeted size range in a given section of the estuary, fewer cages were seeded rather than altering fish density (Table 1). To evaluate somatic growth during the course of the experiment, each juvenile was marked by clipping the dorsal or anal fins, or both. Juveniles were then injected intramuscularly with tetracycline (0.05 mg/kg) to create an otolith mark indicative of the beginning of the caging period. To determine the correct dose for each individual, wet weights were estimated from SL measurements by using a relationship previously obtained for juveniles captured in Punta Banda Estuary (wet weight $(g) = 8.77 - 0.36SL + 0.0045SL^2$, $r^2 = 0.99$).

We were concerned about cage loss. Hence, half the cages were retrieved after 14 days during lower low tide ($n$=2 cages per section of the estuary) and the remainder were recovered after 28 days. Otolith growth rates were thus measured after a 14- or 28-day caging period. Following retrieval from the cages, juveniles were identified by their pattern of fin clippings, SL was measured, and the number of survivors was recorded. Fish were placed on ice in the field and frozen in the laboratory for subsequent analysis of otolith growth rates and gut fullness levels. Otoliths were removed and prepared as described above. It was more difficult to visualize daily growth increments in caged individuals that in those from natural populations (see also Fodrie and Herzka, 2008). Hence, we measured the width of the otolith anterior margin from the tetracycline mark to the edge. Tetracycline marks were viewed under 400× magnification under ultraviolet light with a FlashUV2 flashlight (375 nm). Otolith growth rate was expressed as μm/day. Individual somatic growth rates of caged fishes were calculated as instantaneous growth coefficients ($G$, 1/d) with the folowing equation:

$$G = \left(\ln(SL_t) - \ln(SL_0)\right) / \Delta t ,$$

where $SL_t$ = standard lengths (mm) measured at the beginning of a caging period;
$SL_0$ = standard lengths (mm) measured at end of a caging period;
$\Delta t$ = number of days fish remained within cages.

We used correlation analysis to examine the relationship between otolith and somatic growth rates ($G$) of caged juveniles retrieved after 14 days.

### Gut fullness levels

Our main objectives were to evaluate whether food availability was related to seasonal patterns in recent growth rates and to compare the amount of food ingested by wild-caught and caged California halibut. We assessed the gut fullness level of all juveniles captured in the field ($n$=456) and those retrieved from cages ($n$=214). The complete digestive tract (esophagus, stomach, and intestine) was dissected whole and preserved in 80% ethanol. An index was developed to classify fish by their relative gut fullness: 1) empty; 2) 1–25% full; 3) 26–50% full; 4) 51–75% full; and 5) 76–100% full. Gut fullness assessment was performed by a single person to maintain consistency. The frequency of occurrence of different gut fullness levels for each section of the estuary and sampling period was calculated as a percentage of the total caged or wild-caught fish processed for a given date.

## Results

### Density and environmental variables

Mean density of juveniles ≤200 mm SL ranged from 0.36 ±0.36 to 9.68 ±2.71 fish/1000 m$^2$ (overall mean=3.31 ±1.07 fish/1000 m$^2$, Fig. 2A). It was roughly two times higher (~10 fish/1000 m$^2$) in the innermost section of the estuary than in the central and outer sections (~4–5 fish/1000 m$^2$) during the winter and spring. Although during summer densities were lower than during winter and spring, they were still twice as high in the inner estuary than in the central and outer sec-

tions (~3 vs. 1.5 fish/1000 $m^2$). ANOVA results indicated that density varied significantly as a function of time ($F_{12,154}$=3.97, $P$<0.005) and among sections of the estuary ($F_{2,154}$=4.77, $P$=0.009). The interaction between factors was not significant ($F_{24,154}$=1.33, $P$ =0.154). *Post-hoc* multiple comparison tests indicated that density was significantly higher in the inner estuary (4.55 ±1.02 fish/1000 $m^2$) than in the central section (2.46 ±0.88 fish/1000 $m^2$). In addition, density was significantly higher during winter (November and February) than during most summer months (June through October).

The size distribution of juveniles varied as a function of time and section of the estuary. Recent recruits (<70 mm SL; Kramer, 1991) were relatively abundant throughout the estuary between October 2004 and February 2005, indicating that the peak in estuarine immigration occurred during winter. However, fish 60–80 mm SL were also captured between May 2005 and July 2005. In the outer section, a large proportion (79% of total) of juveniles <100 mm SL were caught between October 2004 and April 2005; few individuals were >200 mm SL (Fig. 2B). Between May 2005 and October 2005, a greater percentage of the fish caught were >200 mm SL (range 21 to 52%, Fig. 2C). In the central and inner sections, 87% and 85% of all fish caught were <200 mm SL. Throughout the estuary, juveniles <200 mm SL predominated between October 2004 and April 2005 (89% of total); fish >200 m SL were more frequent between June2005 and October 2005 (23% of total, Fig. 3).

The size-frequency distributions for the outer and central sections exhibited a single marked peak of 40–90 mm SL (51% of all fish caught) and 40–70 mm SL (32% of all fish caught), respectively (Fig. 4). In contrast, the size-frequency distribution of halibut caught in the inner section had a primary peak at 40–70 mm SL (21% of all fish caught) and a broad, secondary peak at 100–150 mm SL (28 % of the total catch). However, the size-frequency distributions of California halibut did not differ significantly among the outer, central, and inner sections of the estuary (Kruskall-Wallis $H$=1.35, $P$=0.51). The maximum SL found in the outer, central and internal sections of Punta Banda Estuary was 680, 788, and 784 mm, respectively.

Instantaneous measurements of temperature, salinity, DO, and depth taken during trawling varied throughout the year (range 15–24.0°C, 32–38‰, 3–11 mg/L; Fig. 5). The GLM results indicated that density was signicantly and negatively related to temperature ($\beta$=0.59, $F$=5.50, $P$=0.020), sampling time ($F$=2.264, $P$=0.039), and the interaction between temperature and salinity ($\beta$=0.016, $F$=4.906, $P$=0.0208). All other predictor variables were not significantly related to density, although there was a weak negative relationship with salinity ($\beta$=−0.302, $F$=3.274, $P$=0.072). The GLM explained a low proportion of the variance (multiple $r^2$=0.208).

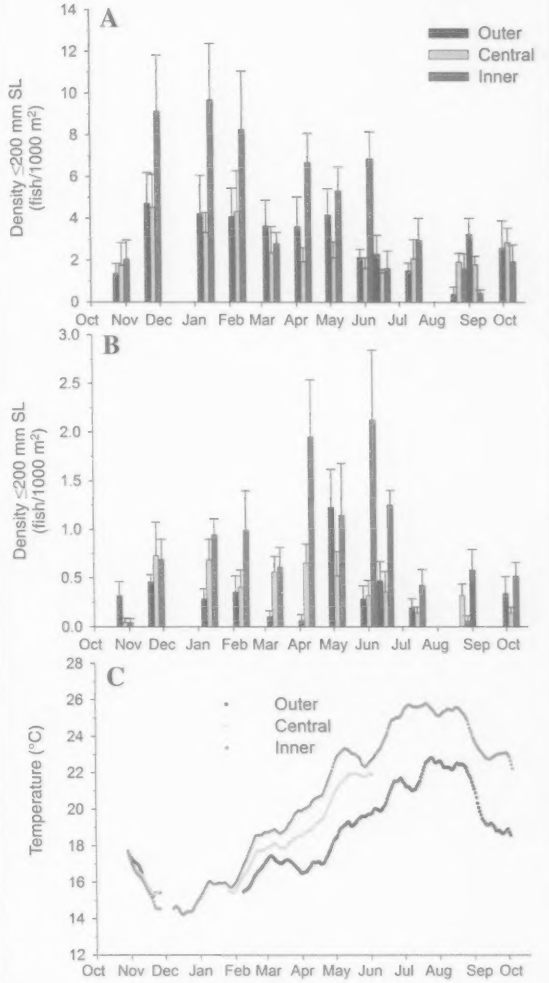


**Figure 2**

(**A**) Density of juveniles ≤200 mm standard length (SL) and (**B**) late juveniles >200 mm SL and adults of California halibut (*Paralichthys californicus)* captured in the outer, central, and inner sections of Punta Banda Estuary between October 2004 and October 2005. Values represent means ± standard error (SE). (**C**) Continuous temperature measurements made at locations in the outer, central and innermost sections of Punta Banda Estuary. Missing data reflect loss of thermographs.

### Otolith growth rates of natural populations

Two-way ANOVA of log-transformed recent otolith growth rates indicated a significant interaction between time and section of the estuary ($F_{10,249}$=8.30, $P$<0.001). In the outer section, the highest mean growth

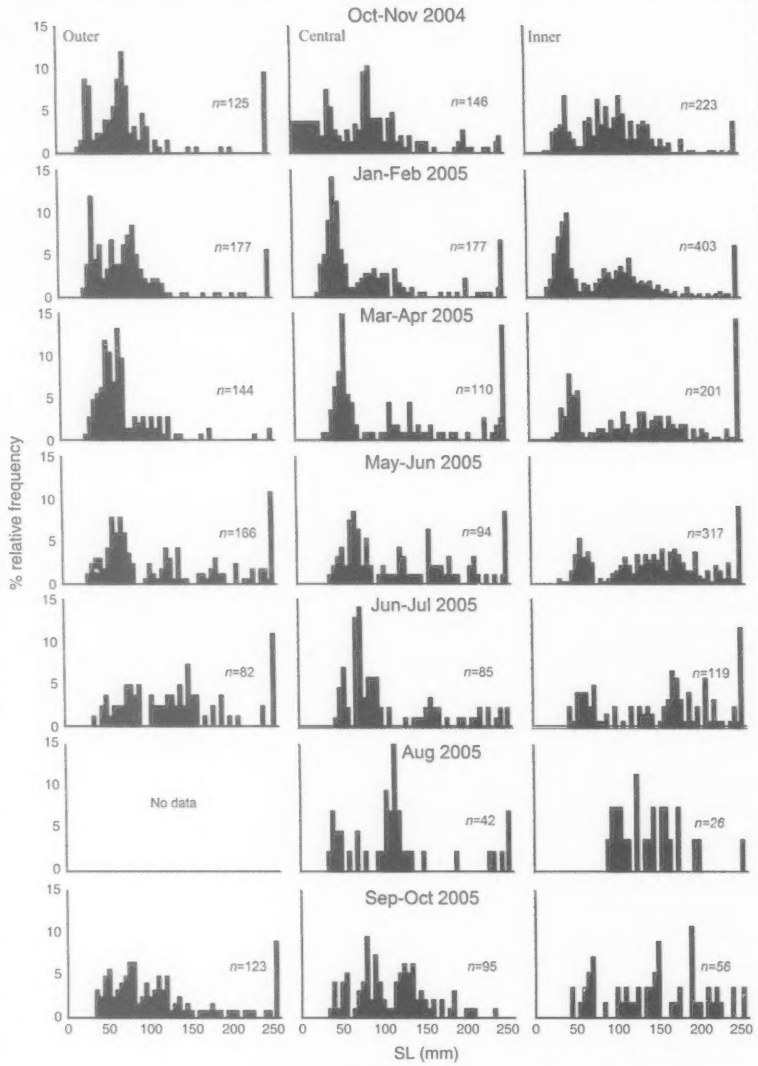


**Figure 3**

Size-frequency distributions of California halibut (*Paralichthys californicus*) captured in the outer, central, and inner sections of Punta Banda Estuary between October 2004 and October 2005. Data corresponding to the beginning and end of each caging experiment were pooled. The 250 mm standard length (SL) size class includes fish ≥250 mm SL.

rates were found in January 2005 and May 2005 (8.13 ±0.40 and 6.75 ±0.45 μm/d, respectively) and the lowest in September 2005 and March 2005 (4.52 ±0.16 and 5.47 ±0.35 μm/d, respectively; Fig. 6A). A similar pattern was observed for the central section. The highest growth rates in the inner section were found in October 2004 and May 2005 (8.40 ±0.32 and 7.78 ±0.41 μm/d, respectively), and the lowest in September 2005 and January 2005 (4.11 ±0.15 and 4.89 ±0.40 μm/d, respectively). Correlation analysis indicated that the relationship between recent otolith growth rates and mean temperature during the 14 days before capture was not significant ($r$=0.31, $F_{1,11}$=1.20, $P$=0.295), even though the mean temperature ranged between 15.8

and 25.7°C (Fig. 2B). There was a positive relationship between density and recent growth rates, but the relationship was not statistically significant ($r$=0.43, $P$=0.074).

### Somatic and otolith growth rates of caged fishes

Throughout the caging experiments, two cages were lost in the outer section after the first two weeks. Another 12 cages were not recovered after 28 days (mostly from the outer section; Table 1). Excluding fish from cages that were not retrieved, 72.7% (131 juveniles) and 72.8% (83 juveniles) were recovered after 14 days and 28 days, respectively.

Recent otolith growth rates during the first 14 days were 5–6 times lower than those of wild-caught California halibut (1–2 μm/d, Fig. 6B). Minimum otolith growth was also detected during the second half of each caging period (Fig. 6C). Somatic growth rates measured after 14 and 28 days were very low, indicating that conditions within the cages did not favor growth (Fig. 6, D and E). We therefore concluded that the growth rates of caged individuals did not reflect habitat value and did not test for growth differences between sections or caging periods.

Despite the low growth rates, there was a significant positive correlation between somatic and recent otolith growth of caged fishes during the first 14 d of the experiments ($r$=0.76, $F_{1,127}$=176.07, $P$<0.001, Fig. 7). This finding indicates that otolith and somatic growth are coupled in California halibut juveniles, even at very low growth rates. There was also a significant negative correlation between somatic growth rates and mean temperature ($r$=0.56, $F_{1,11}$=5.15, $P$ =0.044), although the correlation between otolith growth rates and temperature was not significant ($F_{1,11}$=1.21, $P$=0.295; data not shown).

### Gut fullness levels

Most juveniles captured in the wild had at least some food in their digestive tract (62%, 60%, and 64% of total in the outer, central and inner sections of estuary, respectively; Fig. 8A). The percentage of individuals with gut fullness levels >50% ranged roughly between 40 and 80%, varying as a function of time and section of the estuary. In general, fewer fish exhibited empty guts during the early spring and summer. In contrast, most of the fishes recovered from the cages after 14 days exhibited empty stomachs (Fig. 8B). Only occasionally did some caged individuals have noticeable amounts of food in the digestive tract.

## Discussion

### Density and environmental variables

California halibut ≤200 mm SL were consistently captured throughout Punta Banda Estuary, indicating the entire system is used as juvenile habitat. Based on the relationship between daily otolith increment counts and SL reported by Rosales-Casián (2004) for juveniles caught in northern Baja California, and the age-length relationship reported by Hammann and Ramírez-Gonzáles (1990) for halibut captured in Todos Santos Bay ($TL$ (cm) = 8.98+9.51$Age$ (yr)) fish <180 mm SL are young-of-the-year (see also Haaker, 1975; Kramer, 1991). Maturity occurs at approximately 20–23 cm total length (TL) for males and 38–47 cm TL for females (Haaker 1975; Love and Brooks 1990). Hence, most of our catch was comprised of juveniles, although some adults were also caught.

The distribution of juveniles relative to the mouth of medium-sized estuaries in Baja California and southern California appears to vary among systems. We

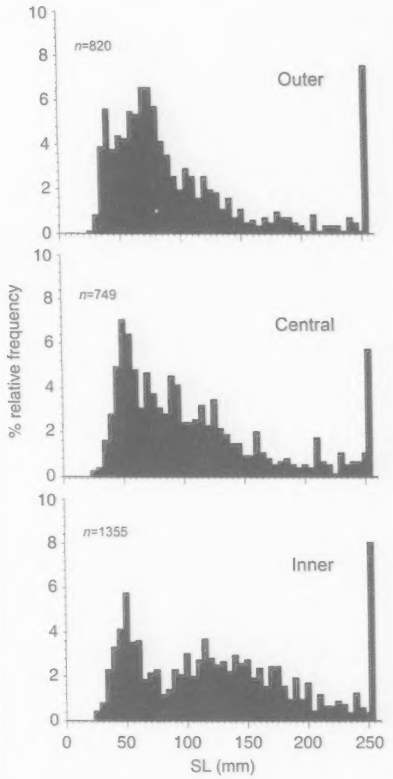


**Figure 4**

Size-frequency distributions of California halibut (*Paralichthys californicus)* captured in the outer, central, and inner sections of Punta Banda Estuary between October 2004 and October 2005. Individuals smaller than 70 mm standard length (SL) are considered recent recruits. The 250 mm SL size class includes fish ≥250 mm SL.

found the highest abundance of juveniles in the inner reaches of Punta Banda Estuary throughout most of the year. Based on an annual survey, Hammann and Ramírez-González (1990) reported higher abundance of juveniles in the central section of the same system between January and April. However, their sampling was limited to the main channel and did not include the inner reaches. Valle et al. (1999) reported that juvenile densities decreased from the mouth to the inner reaches of Alamitos Bay in southern California. In a fall survey, Fodrie and Mendoza (2006) found higher abundance of juveniles in the central and outer reaches of the systems in southern California most similar in size, shape, and depth to Punta Banda Estuary (Batiquitos, Agua Hedionda Lagoon, Buena Vista, and Mission Bay). The density of juveniles captured in Punta Banda estuary is toward the lower range of those reported by Fodrie and Mendoza (2006) for medium-size embayments (termed lagoons in their study, range 4–30 fish/1000m$^2$ after taking into account their gear efficiency corrections).

Juveniles were caught throughout the range of instantaneous temperatures measured in Punta Banda Estuary (15–24°C), although our GLM analysis indicated a weak but significant negative relationship between temperature and density. Likewise, other studies have also reported capturing juveniles at a broad range of temperatures (e.g., Allen 1988; Kramer 1990; Fodrie and Mendoza 2006). Madon (2002) conducted laboratory studies on juveniles ranging from 118–172 mm SL and found them tolerant to a broad range of temperatures (14–28°C) and salinities (8–34‰). Within this size range, growth and osmoregulation were positive at various combinations of temperatures and salinities, except when both were low and outside the range of those measured in this study (14°C and 8‰). Likewise, laboratory studies on California halibut between 40–50 mm TL acclimated to 15°, 18°, 21°, and 24°C indicate preferred temperatures are similar to acclimation temperatures (Esquer-Méndez, 2006). Further, juveniles acclimated to 15–24°C only avoid temperatures substantially above and below those of the acclimation interval (<10.8°C and >29.1°C). The broad temperature interval over which juveniles were captured in this and other studies is thus consistent with the high temperature tolerance of relatively small juveniles.

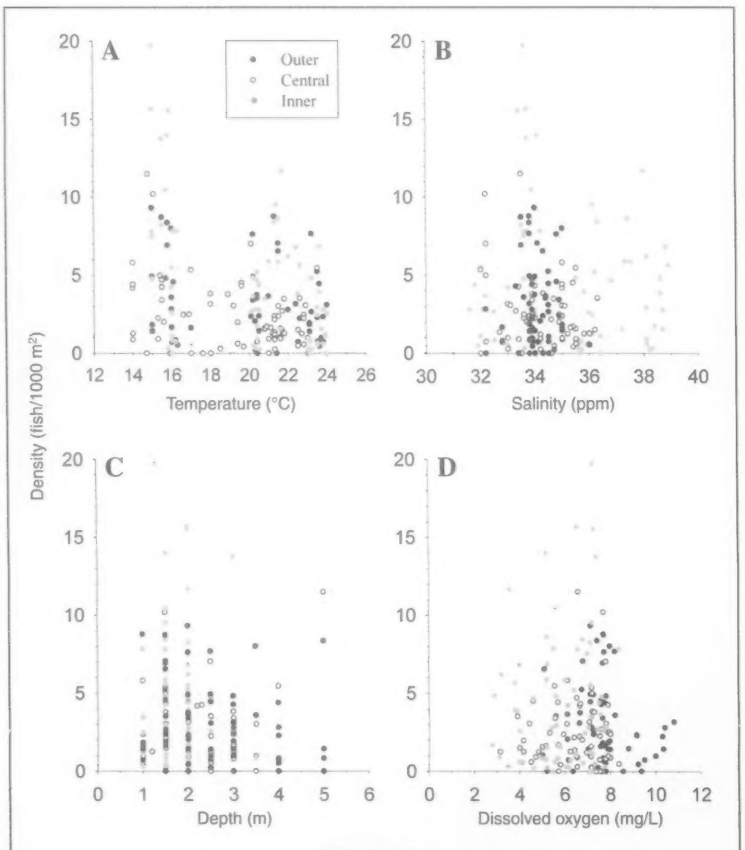


**Figure 5**

Scatterplots of standardized density of California halibut (*Paralichthys californicus*) caught in the outer ($n$=62), central ($n$=65), and inner ($n$=66) sections of Punta Banda Estuary in relation to (**A**) temperature, (**B**) salinity (in parts per million, ppm), (**C**) depth, and (**D**) dissolved oxygen (in mg/L) measurements taken during trawl sampling excursions between October 2004 and October 2005.

In a review of juvenile flatfish distribution in relation to environmental conditions, Gibson (1994) noted that salinity generally has a limited effect on growth of juvenile flatfish, and that its primary effect is on distribution and movement patterns, although others have noted that high salinities may imply a bioenergetic cost to juveniles (Wuenschel et al., 2005). Although the GLM analysis indicated a weak, non-significant relationship salinity and density, juveniles were captured in variable densities over a range of salinities (31.6–38.9‰). Further, we found higher densities of juvenile California halibut in the inner reaches of Punta Banda Estuary, where salinity is usually highest. Hence, it does not appear that the higher salinities we measured during our study limited habitat availability. Given that salinity gradients in seasonally arid estuaries are much more limited than in "classical" systems,

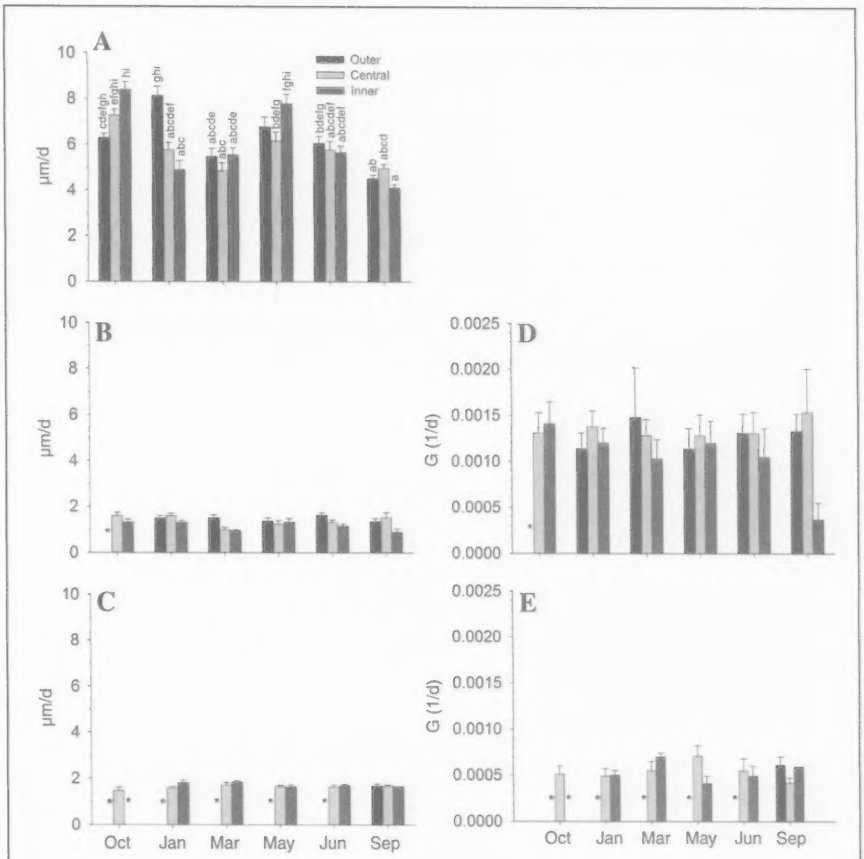


**Figure 6**

(**A**) Recent otolith growth rates of wild-caught juvenile California halibut (*Paralichthys californicus)* sampled between October 2004 and October 2005. Different letters indicate significant differences. (**B–E**) Recent otolith and somatic growth rates of juveniles held in cages for either 14 (**B, D**) or 28 days (**C, E**). Values are means ±standard error (SE). Asterisks represent lost cages.

tolerant species such as juvenile California halibut may utilize the entire embayment as juvenile habitat.

Various studies have reported that small juvenile California halibut are found in higher densities in shallow habitats (~<2 m; Allen and Herbinson, 1990; Fodrie and Mendoza, 2006). This is consistent with our results; the highest densities of juveniles in Punta Banda Estuary occurred at depths between 1–2 m. To our knowledge there are no studies examining the causes of underlying the depth-related distribution patterns in juvenile California halibut, but size-specific depth preferences have been associated with resource partitioning, avoidance of predation by conspecifics, and feeding excursions into shallow areas (Kramer 1990; Gibson, 1994; Able et al., 2005). In addition, the use of shallower habitats subject to stronger variations in temperature by smaller juveniles is consistent with the higher tolerance of smaller juveniles reported by Madon (2002).

The low proportion of the variance explained by the GLM analysis suggests other abiotic or biotic variables also influence juvenile density. The lack of a significant relationship between dissolved oxygen concentrations and density is probably due to the absence of low DO levels during our surveys; values <2 mg/L are typically associated with detrimental effects on growth and survival of fishes (Stickney, 2000). Abundance of juvenile flatfish has been negatively related to sediment grain size, although the relationship varies among species and with size (e.g., Drawbridge, 1990; Amezcua and Nash, 2001). This may be due to the influence of sediment grain size on prey availability, or related to the burial

behavior typically exhibit by flatfish in response to predation pressure or as part of their ambush feeding behavior (Haaker, 1975; Allen, 1990; Amezcua and Nash, 2001). We did not evaluate sediment grain size during our study. However, Ortiz et al., (2003) reported that in Punta Banda Estuary sediment grain size ranges from fine sand (0.19–0.93 mm) near the mouth of the to coarse silt (0.03 mm) near the head. We found that density was generally highest in the innermost reaches of the estuary, where sediments are finest. Hence, the higher abundance of juveniles in the inner estuary may be at least partially related to the characteristics of the substrate.

There is consistent evidence to suggest that the peak in estuarine immigration occurs primarily during winter and spring (Allen, 1988; Kramer, 1990; Hammann and Ramírez-González, 1990; Valle et al., 1999), although settlement can also take place during the summer months (Allen et al., 1990). Based on analysis of the long-term larval surveys preformed as part of the California Cooperative Oceanic Fisheries Investigations Program (CalCOFI), Moser and Watson (1990) reported that off the coast of northern Baja California and in southern California, the peak in larval abundance occurs between February and April, with a secondary peak during summer. We observed the highest abundance of recent recruits (40–50 mm SL) during winter and spring. There was secondary peak of small juveniles during the summer, which could reflect either estuarine recruitment (the recent ingress of juveniles into the system) or growth of previous recruits that entered the estuary at a very small size.

The marked decrease in the density of juveniles >150 mm SL that we observed during the summer is strongly suggestive of estuarine emigration. Although size-selective mortality of larger juveniles (Sogard, 1997) could also lead to a decrease in density, the decrease occurred primarily in the inner and central estuary and was accompanied by an increase the in abundance of larger juveniles in the outer section, which is consistent with movement toward the mouth of the estuary. Given that estuarine emigration coincided with the onset of higher temperatures within Punta Banda Estuary, temperature gradients may provide the cue for emigration.

Lastly, late juveniles >200 mm SL and adults were captured throughout Punta Banda Estuary, albeit in low numbers. Spawning only occurs in coastal areas (Haaker, 1975). The presence of late juveniles and adults within the estuary suggests that these systems may serve as feeding grounds for larger halibut, despite their predominantly coastal habits (Haaker, 1975; Allen, 1990).

### Otolith growth rates of natural populations and gut fullness

The daily otolith growth rates of the fastest (October 2004; 7.32 μm/d) and slowest (September 2005; 4.54 μm/d) growing juveniles 50–160 mm SL varied approximately two-fold during the study. These values

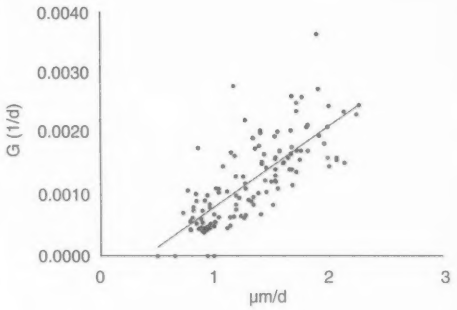


**Figure 7**

Linear regression between somatic and recent otolith growth rates of juvenile California halibut (*Paralichthys californicus*) held in cages for 14 days ($G$ (1/d)=−0.0005 + 0.0013*μm/d, $r$=0.76, $n$=129).

are within the range of daily otolith growth rates reported for juvenile fishes (Sogard and Able, 2002; Gilliers et al., 2006). Recent otolith growth rates varied significantly as a function of time and section of the estuary, although juveniles grew throughout the year. This indicates spatial and temporal variability in the quality of juvenile habitat within the estuary (Sogard, 1992; Phelan et al., 2000; Necaise et al., 2005). Temperature, food availability and quality, size and predation pressure are considered the most important factors influencing growth in juvenile flatfish (Gibson, 1994). We did not find a relationship between the average temperatures and recent otolith growth, although other studies on California halibut indicate that an increase in temperature from 20° to 23°C leads to higher somatic growth rates in the absence of food limitation (Haaker, 1975; Gadomski and Caddell, 1991). This could indicate that temperature does not substantially influence otolith growth rates (Campana and Jones, 1992). However, Kicklighter (1990) reported a significant linear relationship ($r$=0.69, $P$<0.001) between temperature and otolith growth rates in caged juvenile California halibut held at a single site. We found a significant negative correlation between somatic growth rates and temperature in our caged fish, although the growth rates were very low. Hence, although there is evidence to suggest temperature does influence otolith growth in juvenile California halibut, for wild-caught juveniles, other physical or biological factors play a more important role.

California halibut are primarily diurnal, visual feeders (Haaker, 1975). During our study, 40–85% of individuals exhibited some degree of feeding (gut fullness levels >0%). Likewise, Plummer et al. (1983) found that 46% of juveniles and young adults (124–176 mm SL) captured in coastal waters using an otter trawl exhibited empty stomachs. Wertz and Domier (1997) reported that 69% of fish 156–1055 mm TL had empty stomachs.

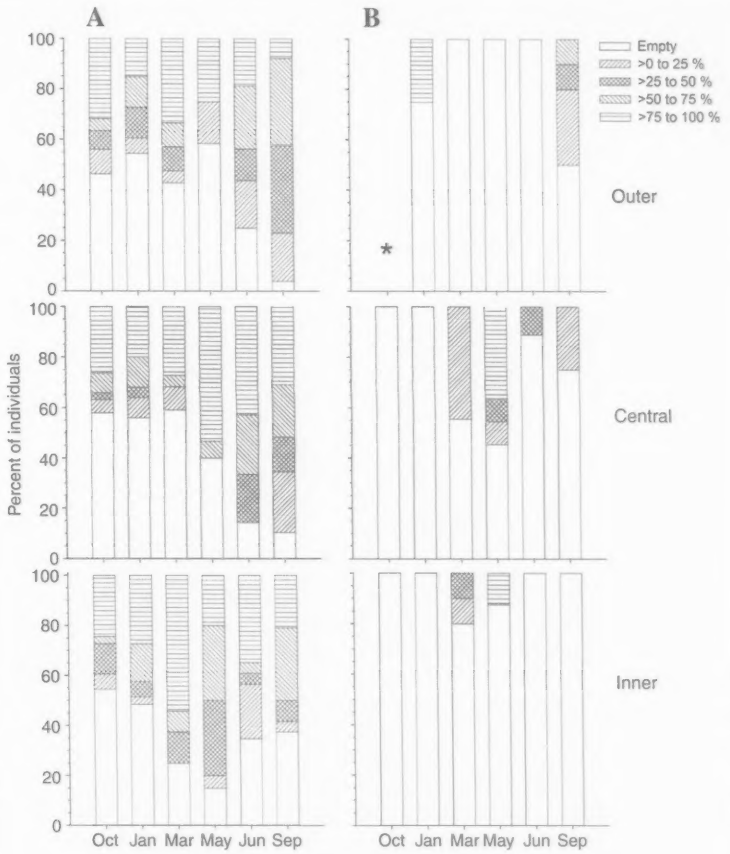


**Figure 8**

Percentage of (**A**) wild-caught and (**B**) caged juvenile California halibut (*Paralichthys californicus)* with different levels of gut fullness. Sampling was performed in the outer, central, and inner sections of Punta Banda Estuary between October 2005 and October 2006. Asterisks represent lost cages.

Regurgitation of gut contents during trawling may partially explain the high incidence of empty stomachs in their studies and ours. However, Plummer et al. (1983) did not find significant diel and spatial differences in gut fullness levels. We have no way of correcting for the potential effect of regurgitation without a full study designed specifically for that purpose. Nevertheless, we find it likely that if regurgitation is frequent in juvenile California halibut, our data are likely to be biased systematically.

Seasonal patterns in gut fullness levels were evident: there was a high (up to 55%) percentage of empty stomachs in all sections of the estuary during the late fall and winter, when temperatures were lowest (~14–18°C). During the spring and summer, when temperatures were substantially higher (18–25°C), the incidence of empty stomachs was substantially lower (10–40%, except for the outer estuary in March). Our qualitative observation that feeding levels are positively related to temperature is consistent with Madon's (2002) laboratory results; he observed an increase in food consumption and metabolic rates at higher temperature. Further, somatic growth rates of juveniles in his experimental treatments (14°, 20°, 25° and 28°C) did not differ significantly, suggesting that higher food consumption is a response to higher metabolic demand.

In contrast, qualitative comparison of the months and sections of the estuary with the highest otolith growth (central and inner sections October 2004, outer section January 2005 and outer and inner sections May 2005) with the proportion of individuals that exhibited evidence of feeding did not show a consistent relationship. Low growth rates (Septem-

ber 2005) did not coincide with a high proportion of empty stomachs. However, our study was not designed to directly relate recent otolith growth with feeding. Gut fullness levels are only a crude point-estimate of feeding success. Our recent otolith growth estimates represent the average daily growth over a 14 day period, whereas the time required to process ingested food is substantially shorter (1–2 days). Detailed studies of food availability and consumption rates (e.g., Sogard, 1992) are necessary to identify the factors underlying variation in otolith growth rates of juvenile California halibut.

Density dependent processes, such as competition for food or space and predation pressure, can also influence growth rates. We found a weak positive relationship between density and recent otolith growth rates of wild-caught juveniles, but the correlation was not significant (see also Fodrie and Levin, 2008). Hence, we did not find strong evidence to suggest limitation for food or space (negative density dependence) or the active selection of habitats supporting to higher growth rates (positive density dependence; Sogard, 1992). However, density-dependent growth regulation may occur at smaller spatial scales than those examined in this study, and cannot be conclusively ruled out.

### Somatic and otolith growth rates of caged fishes

Due to the low growth rates of caged juveniles, we could not use our recent somatic and otolith growth measurements as proxies for habitat quality. Most caged juveniles had empty stomachs. This could be due to a variety of factors, including limited availability of prey within the cages, limited feeding success and foraging ability, competition for food resources due to high fish density, or as a result of a stress response in relation to handling (e.g., Guindon and Miller, 1995). A density of 6 fish/$m^2$ is much higher than densities of juvenile California halibut found within embayments (Fodrie and Mendoza, 2006).

During the first 14 days of caging, somatic growth rates of caged juveniles were very low ($G$=0.0003 to 0.0015 1/d). For fish 50 and 160 mm SL, this is equivalent to 0.01 to 0.12 mm/d, respectively, which is much lower than has been reported previously for wild-caught juveniles California halibut (0.13–1 mm/d, Haaker, 1975; Allen, 1988; Kramer, 1990; Kicklighter, 1990). Accordingly, recent otolith growth rates of caged juveniles were also three to six times lower than in wild-caught juveniles (equivalent to 0.89–1.63 $\mu$m/d, mean=1.32 $\mu$m/d). Kicklighter (1990) also found very low otolith growth rates during his caging experiments; daily increment widths along the main growth axis were 0.49 to 2.26 $\mu$m. Fodrie and Herzka (2008) also reported limited otolith growth in some juveniles held in cages in Punta Banda Estuary.

Somatic and otolith growth rates calculated over the 28 days caging period were substantially lower than over the first 14 days, implying little or no growth during the second half of the experiment. The significant positive relationship between somatic and otolith growth during first 14 days indicates marginal increment widths are reliable proxies for somatic growth at low growth rates, at least for a few days. However, uncoupling between otolith and somatic growth rates occurred during the second half of the experiment; there was virtually no difference in marginal increment widths between fish retrieved after 14 and 28 days. As discussed by Paperno et al. (1997), uncoupling between otolith and somatic growth rates tends to occur under extreme starvation conditions.

## Conclusions

Our results indicate the entire Punta Banda Estuary serves as juvenile habitat for California halibut. This suggests that in arid or seasonally regions in which estuaries lack in strong environmental gradients, particularly in salinity, entire systems may provide suitable habitat for juvenile California halibut. Fodrie and Levin (2008) found evidence to suggest that juvenile abundance is an adequate proxy for recruitment to adult populations in this species. If so, the innermost section of Punta Banda Estuary, in which temperature and salinity is highest during most of the year, may contribute the most to the production of adults. In contrast, Fodrie and Herzka (2008) used otolith microchemistry to evaluate the nursery contribution of juveniles from different sections of Punta Banda Estuary to subadult production in the adjacent coastline, and found that the central and outer sections produced the majority of recruits. Considering that juveniles from the inner estuary must migrate through the central and outer sections of the estuary to emigrate from the system, it is possible that Fodrie and Herzka's (2008) estimates of subadult production are biased. If the abundance of juveniles is indeed a good proxy for production of adults, then the inner section of Punta Banda Estuary is probably the most important area in terms of juvenile California halibut habitat. Put together, these studies highlight the need for assessing juvenile habitat utilization and production on various spatial and temporal scales.

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# Spatial and seasonal relationships between Pacific harbor seals (*Phoca vitulina richardii*) and their prey, at multiple scales

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**Abstract**—Knowing where pinnipeds forage is vital to managing and protecting their populations, and for assessing potential interactions with fisheries. We assessed the spatial relationship between the seasonal distribution of Pacific harbor seals (*Phoca vitulina richardii*) outfitted with satellite transmitters and the seasonal distributions of potential harbor seal prey species in San Francisco Bay, California. Pearson's correlation coefficients were calculated between the number of harbor seal locations in an area of the San Francisco Bay and the abundance of specific prey species in the same area. The influence of scale on the analyses was assessed by varying the scale of analysis from 1 to 10 km. There was consistency in the prey species targeted by harbor seals year-round, although there were seasonal differences between the most important prey species. The highest correlations between harbor seals and their prey were found for seasonally abundant benthic species, located within about 10 km of the primary haul-out site. Probable foraging habitat for harbor seals was identified, based on areas with high abundances of prey species that were strongly correlated with harbor seal distribution. With comparable local data inputs, this approach has potential application to pinniped management in other areas, and to decisions about the location of marine reserves designed to protect these species.

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Identification of foraging habitat is essential to understanding the ecology of marine predators. This information is vital to managing and protecting populations, as well as assessing the potential effects of commercial and recreational fisheries on both the marine predator and the fisheries. We determined the spatial overlap of one marine predator, the Pacific harbor seal (*Phoca vitulina richardii*) and its prey in the San Francisco Bay estuary (SFB), California, in order to identify foraging areas and seasonal patterns of resource use by this coastal pinniped.

The Pacific harbor seal (hereafter referred to as the harbor seal) is a small phocid seal common to waters along the west coast of North America. Harbor seals are opportunistic predators, feeding primarily on benthic species and small, epibenthic, schooling fishes, and occasionally foraging on pelagic species (Harkonen, 1987). A relatively small number of species tend to dominate the diet of harbor seals, but seasonal shifts in diet are seen in many areas, associated with seasonal fluctuations in prey availability (Brown and Mate, 1983; Tollit et al., 1998). Fecal samples collected in SFB indicate that harbor seals in this region feed on Pacific herring (*Clupea pallasii*), northern anchovy (*Engraulis mordax*), plainfin midshipman (*Porichthys notatus*), Pacific staghorn sculpin (*Leptocottus armatus*), white croaker (*Genyonemus lineatus*), yellowfin goby (*Acanthogobius flavimanus*), jacksmelt (*Atherinopsis californiensis*), and English sole (*Pleuronectes vetulus*) (Torok, 1994). Young harbor seals have a reduced diving capability, and eat benthic crustaceans—primarily shrimp (e.g., *Crangon* spp.) (Bigg, 1973). Based on VHF (very high frequency) radiotelemetry tracking, the foraging range of harbor seals in SFB is mainly within 1–5 km of a haul-out site (Torok, 1994; Nickel, 2003), indicating that harbor seals in SFB feed on local prey. Abundance of prey and distance from the primary haul-out site are the strongest predictors of harbor seal use of an area in SFB (Grigg, 2008).

Given information on what harbor seals in the region were eating, our primary goal was to identify where these harbor seals were foraging within the study area. Identifying the preferred foraging habitat of pinnipeds can be difficult because of the logistical challenges associated with locating and tracking these large, highly mobile animals while they are at sea. Harbor seals are considered "central place foragers" (Orians and Pearson 1979) in that they return to a central place (the haul-out site) after foraging trips, they repeatedly visit specific foraging areas, and they tend to focus their foraging effort in areas near the haul-out site (Thompson et al., 1998). Radiotracking, both conventional VHF and satellite-linked (hereafter referred to as satellite tracking), is used to identify areas used by tagged animals, and to measure the frequency with which animals return to these areas. For central-place foragers who feed primarily on a limited number of prey species and exhibit site fidelity to specific foraging areas, telemetry can be used in conjunction with information on prey distribution to identify foraging habitat (North and Reynolds, 1996; Robinson et al., 2007). The harbor seal was therefore an excellent subject with which to assess the usefulness of telemetry in identifying foraging habitat of pinnipeds.

A second goal of this study was to assess the influence of spatial scale on our analysis. A number of investigators have assessed the spatial overlap between distributions of marine predators and their prey (e.g., Rose and Leggett, 1990; Fauchald et al., 2000; Davoren et al., 2003). These investigators noted that the results of such analyses vary depending on scale (Rose and Leggett, 1990; Fauchald et al., 2000). As a result, studies encompassing multiple scales are recommended for addressing questions related to habitat selection (Olivier and Wotherspoon, 2005).

Although harbor seals in SFB appear to forage within the bay, harbor seals do periodically make trips to the outer coast (Grigg, 2008). A third goal of this study was to attempt to identify environmental factors associated with harbor seals leaving SFB to forage. Harbor seals could forage outside of SFB to exploit changes in availability of prey associated with coastal upwelling. Upwelling of cooler, nutrient-rich water is associated with increased productivity along the California coast and can influence the distribution of marine predators (Becker and Beissinger, 2003). Alternatively, harbor seals could forage outside of SFB when high numbers of harbor seals using haul-out sites within SFB result in intraspecific competition for food resources in SFB.

We examined the associations between harbor seals and potential prey species, using satellite tracking to identify patterns of harbor seal distribution in SFB and a database available from the California Department of Fish and Game (CDFG) on abundance and distribution of potential prey species in the area. The objectives of this study were 1) to identify spatial and seasonal patterns of association between harbor seals and their prey in an urbanized estuary; 2) to examine the influence of scale of analysis on the spatial relationship between predator and prey; 3) to relate shifts in prey abundance or environmental factors to seasonal differences in the use of waters inside and outside SFB; and 4) to assess the usefulness of satellite telemetry in identifying foraging areas of harbor seals.

## Materials and methods

### Study area

San Francisco Bay is a turbid estuary with mean depths ranging from 3–11 m, and is the largest coastal embayment on the Pacific coast of the United States (Conomos et al., 1985) (Fig. 1). Harbor seals use SFB year-round for foraging, pupping, and resting on terrestrial haul-out sites (Allen et al., 1993; Grigg et al., 2004).

### Harbor seal telemetry

Between January 2001 and January 2005, harbor seals were captured at a primary haul-out site in SFB (Castro Rocks; Fig. 1). At high tide, "tangle nets" 20–40 m long and 5 m deep were set, and harbor seals were caught as they approached the site. Harbor seals were fitted with dorsal- or head-mounted satellite-linked Platform Terminal Transmitters (PTTs; model ST-18, Telonics, Mesa, AZ; models SDR-T16 and SPOT3, Wildlife Computers, Redmond, WA; depending on model, tag power ranged from 0.4 to 0.5 watt, repetition rate ranged from 45 to 48 sec). Only harbor seals deemed large enough (≥40 kg) to support the PTTs were tagged. The PTTs were glued to the harbor seal's pelage with a quick-setting marine epoxy, and were shed by harbor seals before or during their annual molt.

Service Argos (CLS America, Inc., Largo, MD) was the processing center for the satellite telemetry data and provided the geographical coordinates of the tagged harbor seals. When a harbor seal is at the surface, PTTs send periodic radio transmissions which are detected by polar-orbiting satellites. These satellites relay the signals to processing centers, where animal location estimates are calculated on the basis of the Doppler effect. Based on the number of received transmissions and other factors, all locations are grouped into location accuracy "classes," ranging from zero to three, and two additional classes (A and B) for locations that could not be assigned an accuracy estimate (Table 1). Marine mammals are considered to be good study animals for satellite tracking because they surface to breathe and this allows sufficient time for a position to be determined by the satellites. In addition, the elevation of the tag does not change while the tag is on the animal; changes in tag elevation have been cited as a primary cause of spatial inaccuracy (Keating et al., 1991). Recent studies have assessed the usefulness of PTTs for studying movements and habitat use of marine animals (e.g., Vincent et al., 2002; White and Sjoberg, 2002). Location classes with lesser accuracy

are customarily removed from the data set, and filtering methods are used to remove improbable locations and improve the mean accuracy of the remaining locations. Vincent et al. (2002) and Hays et al. (2001) found that locations in classes B and 0 were inaccurate, but class A locations were more accurate and comparable to class 1 locations (Table 1). We therefore removed location estimates in classes B and 0 from the record of each harbor seal's movements, but did not automatically remove locations in class A. All points that fell on land were removed, and then the remaining locations were filtered according to the speed necessary for a harbor seal to move between two successive locations, calculated in a geographic information system (GIS). Any location that would have required a travel speed greater than 10 km/hr, or 2.78 m/s (Lowry et al., 2001),was flagged for inspection. These questionable harbor seal locations were assessed by their spatial and temporal relationship to the prior or subsequent location with a greater accuracy rating, and unlikely locations were removed. Points that would have required an isolated movement away from and immediately returning to the same area, necessitating a narrow V-shaped movement track, were also eliminated (see Keating, 1994). Locations within 1 km of a haul-out site were removed in order to eliminate locations associated with haul-out site use or underwater movements unrelated to foraging (Thompson and Miller, 1990). Finally, for these analyses, we analyzed spatial overlap between harbor seal locations and abundance of potential prey species around SFB, rather than analyzing sequential tracks of movement by individual harbor seals. To improve independence of point location estimates for the correlation analyses, locations recorded within one hour of another location for the same harbor seal were removed from the data set.

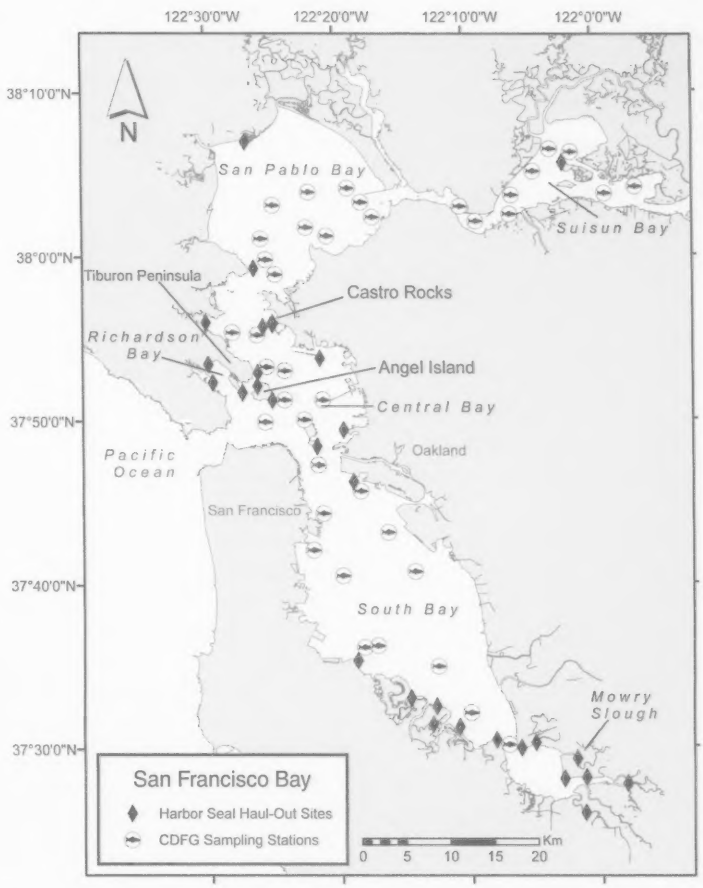


**Figure 1**

The San Francisco Bay, CA, study area, showing Castro Rocks and other primary harbor seal (*Phoca vitulina richardii*) haul-out sites, and 39 fish sampling stations around the bay sampled by the California Department of Fish and Game(CDFG). Catch-per-unit-of-effort data from the CDFG sampling stations were used to build seasonal harbor seal prey distribution maps for San Francisco Bay. Coastline data layer created by NOAA/NOS/ORR/CPRD (available online).

### Correlation analyses

Records of prey distribution and abundance during the study period were obtained from the Interagency Ecological Program for the San Francisco Estuary and from the San Francisco Bay Study, California Dep. Fish and Game. Monthly samples of fish, crab, and shrimp species were collected by CDFG at 39 sampling stations located around SFB (Fig. 1), using two sampling methods: an otter trawl (OT) and a midwater trawl (MWT). The OT was used to sample bottom-dwelling fish, shrimp, and crab, the MWT was used to sample mid-water fish, and both trawls yielded quantitative data on fish abundance. For this study, we focused on eight species of fish known to be prey of harbor seals in SFB, combined crab species (primarily Dungeness crab, *Cancer magister*), and combined shrimp species (primarily *Crangon* spp.). Given concerns about harbor seals foraging on salmonids, CDFG data on the abundance and distribution of Chinook salmon (*Oncorhynchus tshawytscha*) in SFB were included in the analysis. Catch per unit of effort (CPUE) was calculated as follows for each station, month, and species, by using gear-specific formulas from CDFG:

$$OT\ \text{CPUE} = (\textit{number caught/tow area}) \times 10{,}000, \quad (1)$$

**Table 1**

Location-accuracy classes for marine mammal location estimates obtained with satellite telemetry and assigned by Service Argos, and reported in other studies. Also included are the proportions of the final, filtered harbor seal *(Phoca vitulina richardii)* location data set from the present study that fell into each location class. Vincent et al.(2002) calculated accuracy for tags deployed on captive grey seals (*Halichoerus grypus*) in France and calculated accuracy separately for the latitude and longitude of position estimates. Hays et al. (2001) calculated accuracy using tags located in fixed positions in Brazil and on Ascension Island, as mean deviation from true tag location.

| Location accuracy class | Proportion of total locations used in this study | Service Argos accuracy estimate (m) | Vincent et al. 2002 (unfiltered; lat./long.) (m) | Hays et al. 2001 (mean deviation from true) (m) |
|---|---|---|---|---|
| 3 | 0.20 | <150 | 157/295 | 270 |
| 2 | 0.25 | <350 | 259/485 | 540 |
| 1 | 0.21 | <1000 | 494/1021 | 1330 |
| 0 | 0 | >1000 | 2271/3308 | 10,100 |
| A | 0.34 | None assigned | 762/1244 | 990 |
| B | 0 | None assigned | 4596/7214 | 7000 |

where *tow area* = distance towed (in meters) × door spread of tow (3.42 m); and

$$MWT \text{ CPUE} = (number\ caught/tow\ volume) \times 10{,}000, \quad (2)$$

where *tow volume* = number of flowmeter revolutions × 0.0269 m/rev × net mouth area (10.7 $m^2$ in this case).

$$Crab/shrimp \text{ CPUE} = \text{number caught per 5 minute tow}, \quad (3)$$

CPUE from the 39 sampling stations was used to create maps of the relative abundance of harbor seal prey species in SFB, by using the inverse distance weighting interpolation method (Geostatistical Analyst extension to ArcGIS 9.2, ESRI, Redlands, CA). Inverse distance weighting is a deterministic interpolation method and makes no assumptions about the input data; this was important given the patchy nature of fish distributions in SFB. Given seasonal differences in prey species' abundance and distribution, and in harbor seal behavior related to breeding and molting, we created four maps for each prey species, one for each harbor seal "season" (spring: March–May; summer: June–August; fall: September–November; winter: December–February). In SFB, harbor seals pup during the spring and molt during the summer. Only records for those months and years when we had tagged harbor seals active in SFB waters were included in the analyses.

Using the Hawth's analysis tools extension (available online at http://www.spatialecology.com/htools) for ArcGIS 9.2 (ESRI, Redlands, CA), a 1-km grid was laid over a map of the entire study area, consisting of all waters from the mouth of SFB, to the eastern edge of Suisun Bay (Fig. 1). All harbor seal locations and environmental data sets in the GIS were reprojected to Universal Transverse Mercator (UTM) coordinates, using the North American Datum of 1927 (NAD 27), zone 10N, and resampled to an initial grid resolution of 1-km. For each season, an average CPUE of each prey species was assigned to each 1-km grid cell, by using the area-weighted mean of the values falling within that grid cell. In addition, we counted the number of harbor seal locations falling within each grid cell; because the number of tagged animals was limited, data from individual harbor seals were pooled for this analysis (see Erickson et al., 2001). The minimum scale of analysis was 1 $km^2$, well within the estimated average accuracy of the filtered harbor seal location data (Table 1; see also Bekkby et al., 2002).

To vary the scale of analysis, data from the 1-km grid cells were combined into progressively larger grid cell sizes, ranging from 2 to 10 km. Given the size of SFB (and the fact that the sample size decreased with each successively larger grouping), we did not consider scales larger than 10 km. Because of the irregular shoreline of SFB, some grid cells overlapped land; therefore, we removed grid cells that represented primarily land from the analyses. For all remaining grid cells, we calculated the number of harbor seal locations per $km^2$.

For each spatial scale (1 to 10 km) and each season, we calculated the Pearson's correlation coefficient between the number of harbor seal locations per grid cell and the CPUE for each potential prey species in that cell. We plotted correlation coefficients versus scale for each season to assess the effects of scale on the strength of the spatial relationships between harbor seals and potential prey.

To estimate the availability of foraging habitat during each season, we used regression tree analyses (Breiman et al., 1984) to identify threshold values of prey CPUEs that would most strongly differentiate between grid cells with greater use by harbor seals and cells with lesser use by harbor seals, for each season. In other words, this threshold value indicated the minimum CPUE for prey in the grid cells representing areas that were fre-

**Table 2**

Harbor seals (*Phoca vitulina richardii*) captured in San Francisco Bay, CA (2001–05) and tagged with satellite telemetry tags, with sample size information. Ages are abbreviated as A (adult), SA (subadult), and Y (yearling). Dates and duration (in days) of telemetry tag attachment are shown. Numbers of both raw seal location estimates and filtered location estimates are shown. Accuracy filtering methods were based on location quality rankings provided by Service Argos, and a spatial filtering mechanism to reduce improbable locations. Location filtering consisted of the removal of points that fell within 1 km of a haul-out site, or outside of the study area.

| Harbor seal ID | Age | Sex | Dates of tag attachment | Days tagged | Number of raw location estimates | Final sample size after accuracy and location filtering |
|---|---|---|---|---|---|---|
| 15345 | A | M | 1/2001–6/2001 | 153 | 307 | 141, 21 |
| 15440 | SA | F | 7/2001–8/2001 | 34 | 106 | 65, 33 |
| 15436 | SA | F | 7/2001–8/2001 | 31 | 69 | 42, 24 |
| 19580 | A | F | 7/2001–3/2002 | 233 | 947 | 547, 365 |
| 19582 | A | M | 7/2001–8/2001 | 22 | 157 | 81, 3 |
| 15439 | A | F | 1/2001–9/2001 | 69 | 204 | 97, 27 |
| 15437 | A | M | 1/2002–5/2002 | 126 | 616 | 188, 26 |
| 10024 | A | F | 8/2002–1/2003 | 158 | 561 | 206, 87 |
| 10278 | A | M | 8/2002–11/2002 | 85 | 45 | 22, 8 |
| 10279 | A | M | 8/2002–11/2002 | 97 | 353 | 133, 63 |
| 10280 | A | M | 8/2002–3/2003 | 229 | 1013 | 451, 218 |
| 10297 | SA | M | 8/2002–3/2003 | 215 | 1156 | 480, 250 |
| 10863 | SA | F | 8/2002–2/2003 | 190 | 1269 | 588, 336 |
| 42526 | SA | F | 8/2003–1/2004 | 141 | 896 | 308, 111 |
| 42527 | A | F | 8/2003–3/2004 | 204 | 1369 | 485, 274 |
| 42529 | Y | F | 8/2003–12/2003 | 107 | 986 | 397, 270 |
| 42530 | Y | F | 8/2003–12/2003 | 123 | 1012 | 372, 202 |
| 21454 | A | F | 1/2005–6/2005 | 144 | 577 | 236, 53 |
| 42528 | SA | M | 1/2005–6/2005 | 135 | 524 | 179, 49 |

quently visited by harbor seals; lower abundances were found in cells repesenting areas less frequently visited by harbor seals. Grid cells with CPUE values above the threshold values identified in the regression tree were designated as potential harbor seal foraging habitat. Harbor seal habitat was mapped by using only the prey species with the highest correlations with harbor seal distribution (correlation coefficient >0.7) at the 10-km scale, because this coarser scale may be more appropriate for assessing behavior which influences lifetime fitness (Rettie and McLoughlin, 1999). The area (in $km^2$) of potential foraging habitat was then calculated, both for SFB as a whole, and within 10 km of the primary haul-out site, Castro Rocks.

To examine potential reasons why some harbor seals were leaving the bay, correlation coefficients were calculated between the proportion of harbor seal locations located outside of SFB, for each season, and the following three variables: 1) prey CPUE inside SFB; 2) number of harbor seals inside SFB and at Castro Rocks; and 3) indices of upwelling measured along the adjacent outer coast. The number of harbor seals in SFB fluctuates seasonally (Fancher, 1979; Grigg et al., 2004; Grigg, 2008), and we hypothesized that an increased number of excursions outside SFB would be positively correlated with the increased number of harbor seals in SFB. To ascertain the numbers of harbor seals in SFB and at Castro Rocks, harbor seals were counted at the three largest haul-out sites in SFB and the numbers were averaged by season across years. For information on the strength of upwelling along the California coast, we used monthly upwelling indices compiled by the National Oceanographic and Atmospheric Administration Environmental Research Division. Statistical significance for all correlations was assessed with $\alpha$=0.05.

## Results

### Harbor seal telemetry

Nineteen harbor seals were captured between January 2001 and January 2005 (Table 2). Data filtering for accuracy reduced the number of location estimates for individual harbor seals by 39–69%, and additional location estimates were removed when within 1 km of a haul-out site, or outside of SFB. The mean number of locations per harbor seal after all filtering was 120 ±27 (standard error of the mean; SEM). The number of locations per season ranged from 134 during the spring season, to 1139 during the fall. Locations were evenly dispersed between day and night (47% during the day, 6 a.m. to 6 p.m., and 53% during the night, 6 p.m. to 6 a.m.).

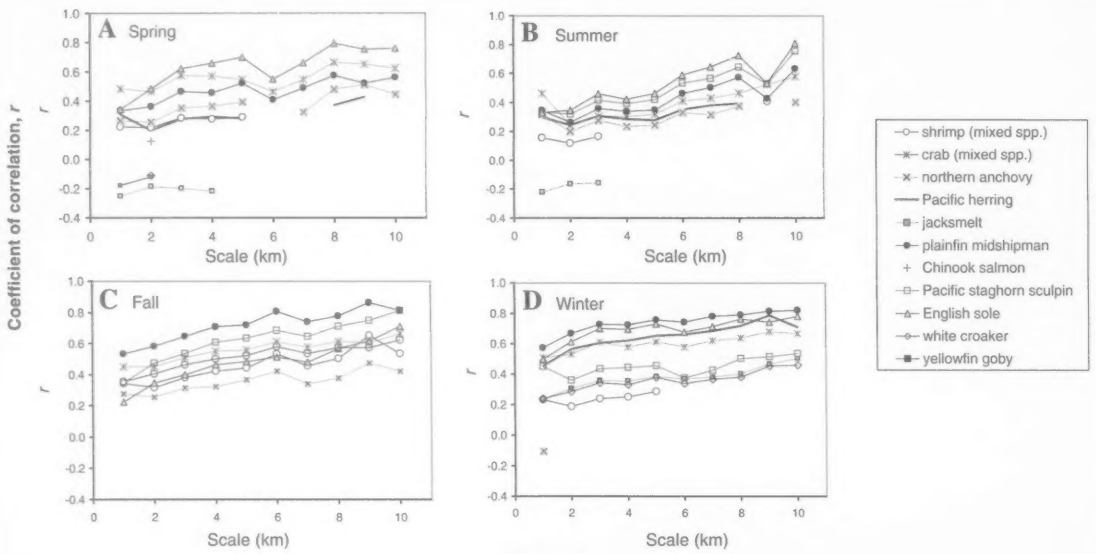


**Figure 2**

The effects of scale on the analyses of spatial overlap between distribution of Pacific harbor seals (*Phoca vitulina richardii*) and their prey species in San Francisco Bay, CA, 2001–05. Potential prey species included Pacific herring (*Clupea pallasii*), northern anchovy (*Engraulis mordax*), plainfin midshipman (*Porichthys notatus*), Pacific staghorn sculpin (*Leptocottus armatus*), white croaker (*Genyonemus lineatus*), yellowfin goby (*Acanthogobius flavimanus*), jacksmelt (*Atherinopsis californiensis*), English sole (*Pleuronectes vetulus*), Chinook salmon (*Oncorhynchus tshawytscha*), shrimp (mixed species, primarily *Crangon* spp.), and crab (mixed species, primarily Dungeness crab, *Cancer magister*). Graphs show the change in strength of Pearson's correlations ($r$) between seal and prey locations with changes in analysis scale from 1 km to 10 km, for each season. Only statistically significant ($P<0.05$) correlations are shown.

### Correlation analyses

According to our analyses, three benthic species—plainfin midshipman, English sole, and Pacific staghorn sculpin—are important prey of harbor seals in SFB (Fig. 2). Spatial overlap between distribution of harbor seals and abundance of primary harbor seal prey species was greatest in central SFB, and in waters around Castro Rocks (Fig. 3). Although harbor seals used areas in the north and south SFB in all seasons, waters of central SFB and surrounding the primary haul-out site, Castro Rocks, were used most frequently. Correlations revealed both year-round consistency in the identity of harbor seal prey species and seasonal differences between primary prey species.

**Spring** (March–May): During the spring pupping season, harbor seal locations were most highly correlated with English sole and crab (Figs. 2A and 3A). In all seasons, there were strong correlations between the distribution of crab and harbor seals (Fig. 4). In contrast to the fall and summer, there was no significant correlation between harbor seal locations and abundance of Pacific staghorn sculpin during the spring.

**Summer** (June–August): During the summer molting season, harbor seal locations were most highly correlated with English sole and Pacific staghorn sculpin (Figs. 2B and 3B). Harbor seals in SFB frequently visited areas where high numbers of Pacific staghorn sculpin were found in the 2001–05 CDFG trawls, most notably in the waters around the Castro Rocks haul-out site.

**Fall** (September–November): During the fall, harbor seal locations were most highly correlated with plainfin midshipman, white croaker, and Pacific staghorn sculpin (Figs. 2C and 3C). Across spatial scales, correlations with plainfin midshipman were particularly strong (often $\geq 0.8$) during the fall.

**Winter** (December–February): During the winter, harbor seal locations were most highly correlated with plainfin midshipman, English sole, and Pacific herring (Figs. 2D and 3D). Harbor seals foraged in Pacific herring spawning areas, and correlations between Pacific herring and harbor seals were greatest during this season. No significant correlation was found between the distribution of harbor seals and Chinook salmon. There was little correlation between the frequency of use of an area by harbor seals and the distribution of yellowfin goby, a non-native species that was found to be an important prey species in an earlier study of the diet of SFB harbor seals (Torok, 1994).

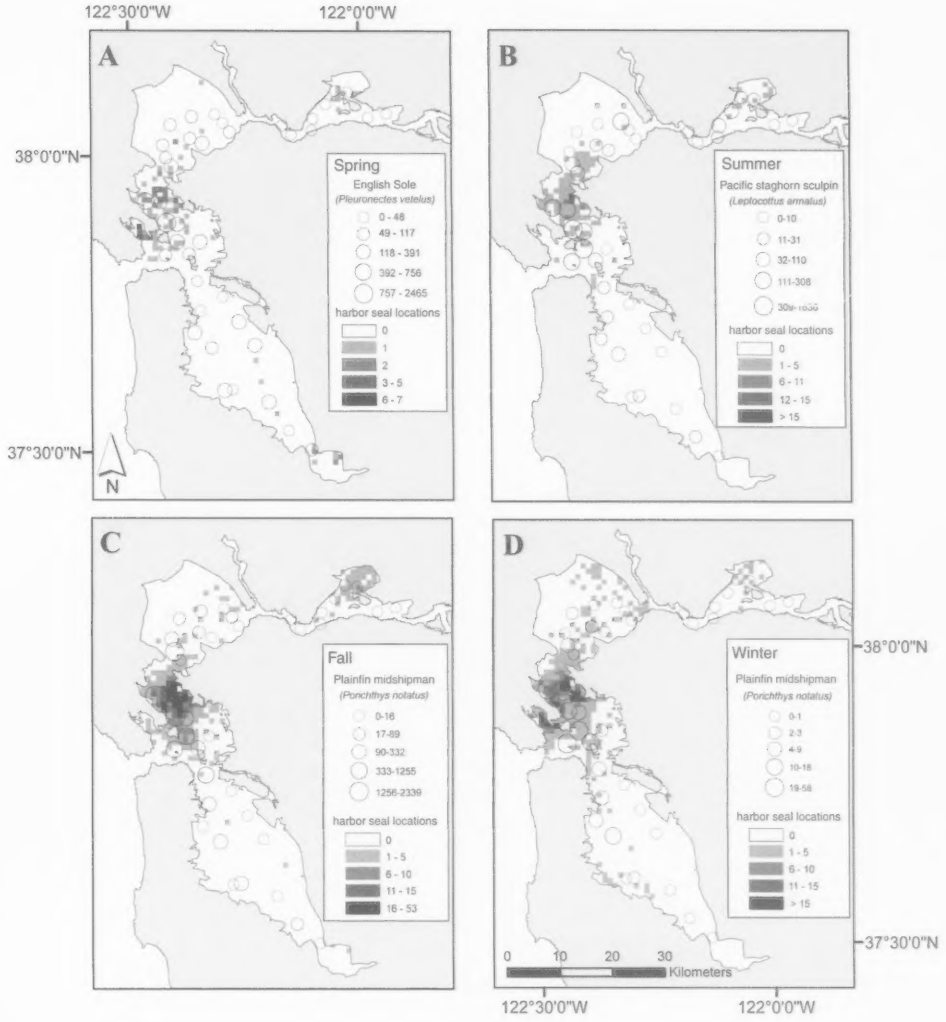


**Figure 3**

Spatial overlap of Pacific harbor seals (*Phoca vitulina richardii*) and important harbor seal prey species in San Francisco Bay, CA, for four seasons, 2001–05. Density of seal locations is indicated by shading of the 1-km grid, with darker shading indicating more frequent use by seals. Relative abundance of specific harbor seal prey species (as noted in the map legends) caught at each fish sampling station is indicated by size of the circle marking station location, and larger circles indicate greater catch per unit of effort of prey at that location, in number of individuals per hectare (1 hectare=0.01 $km^2$). Fish species shown are representative of harbor seal prey species found to have the greatest correlation with seal location for that season: **A**) Spring—English sole (*Pleuronectes vetulus*), **B**) Summer—Pacific staghorn sculpin (*Leptocottus armatus*), **C**) Fall—plainfin midshipman (*Porichthys notatus*), and **D**) Winter—plainfin midshipman (*P. notatus*).

At the 1-km scale, most correlations were positive (i.e., greater co-occurrence of harbor seal locations with greater prey CPUE), but not strong (correlation coefficients <0.6). The correlation coefficients for plainfin midshipman were greatest among most spatial scales during the fall and winter. The coefficients for English sole were greatest among most spatial scales during the spring and summer. The coefficients for Pacific staghorn sculpin were second greatest during summer and fall.

In general, correlations increased with scale (Fig. 2). At the largest scale (10-km), correlations between distribution of harbor seals and primary prey CPUEs were often ≥0.8. The prey species with high correlations (>0.7) at the 10-km scale included plainfin midshipman (fall and winter), Pacific staghorn sculpin (summer and fall), English sole (all seasons), and Pacific herring (winter) (Fig. 2). During spring, only English sole had a correlation of >0.7. For the foraging habitat map for spring, therefore, we included crab, which most closely approached the 0.7 threshold value. Based on the threshold prey abundances identified in the regression tree analysis, potential foraging habitat available in SFB ranged from 147 $km^2$ in spring to 238 $km^2$ in fall (Table 3, Fig. 5). Foraging habitat available within 10 km of Castro Rocks ranged from 101 $km^2$ in spring to 144 $km^2$ in fall.

When assessing seasonal differences in harbor seals' use of waters inside vs. outside SFB, the proportion of harbor seal locations on the outer coast was greater during the summer (0.33) and spring (0.21) than during the fall (0.01) or winter (0.08). Use of areas outside of SFB was not correlated with prey CPUE in SFB, number of harbor seals in SFB or at Castro Rocks, or upwelling indices. In general, the proportion of harbor seal locations recorded outside of SFB decreased with greater levels of prey availability in SFB, increased with greater numbers of harbor seals in SFB as a whole, and increased with greater upwelling indices. In contrast, when the proportion of locations on the outer coast was compared with the average maximum count at Castro Rocks, the proportion on the outer coast tended to be lesser when numbers of harbor seals at Castro Rocks were greater.

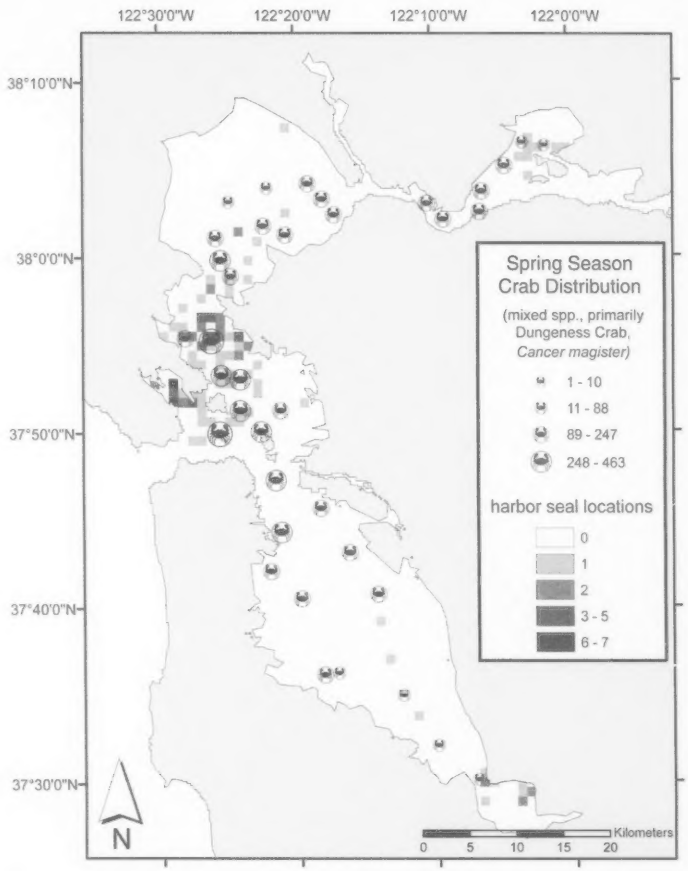


**Figure 4**

Spatial overlap of Pacific harbor seal (*Phoca vitulina richardii*) distribution with abundance of crab species (mixed species; primarily Dungeness crab, *Cancer magister*) in San Francisco Bay, CA, during the spring seal pupping seasons, 2001–05.

## Discussion

We used a simple approach for identifying harbor seal foraging areas, using satellite tracking, available information on harbor seal diets from previous studies, and a data set on prey distribution obtained from a local management agency. In many cases, the abundance and distribution of prey is the most important factor influencing the spatial distribution of predators (Davoren et al., 2003). The primary motivation for a predator to move is to locate prey patches which offer a sufficient energetic "reward" (Charnov, 1976), i.e., provide sufficient energy gained from ingestion of prey, once the energetic costs of capture have been deducted. Not surprisingly, both terrestrial and marine mammals have been found to choose habitats that provide resources necessary for survival and reproduction, and for this reason, use of an area is often assumed to reflect quality or abundance of resources available in that area (Boyce and McDonald, 1999; Davoren et al., 2003).

Despite some controversy about possible biases in fecal analyses used to estimate diets (Harvey, 1989; Tollit et al., 1998), analysis of fecal samples remains an important method of identifying prey species of pinnipeds. However, it is often difficult to identify where harbor seals are foraging, even when fecal analyses are supplemented by VHF telemetry tracking (Torok, 1994). This problem has led some researchers to use techniques such as fatty acid signatures (Iverson et al., 1997) to identify harbor seal foraging areas. Our study presents an alternate approach to identifying these areas.

**Table 3**

Area ($km^2$) of Pacific harbor seal (*Phoca vitulina richardii*) foraging habitat in San Francisco Bay, CA, based on areas where abundance of harbor seal prey species was above the threshold value calculated in the regression tree analyses. Area of foraging habitat throughout SFB is given, as well as area of foraging habitat within 10 km of Castro Rocks, the primary haul-out site used by harbor seals in this study.

| Season | Prey species used in estimation of foraging habitat | Area of foraging habitat ($km^2$) | Area of foraging habitat within 10 km of Castro Rocks ($km^2$) |
|---|---|---|---|
| Spring | English sole (*Pleuronectes vetulus*)<br>Crab (mixed spp.; primarily Dungeness crab, *Cancer magister*) | 146.8 | 100.7 |
| Summer | Pacific staghorn sculpin (*Leptocottus armatus*)<br>English sole | 222.9 | 140.2 |
| Fall | Plainfin midshipman (*Porichthys notatus*)<br>Pacific staghorn sculpin<br>English sole | 238.4 | 144.1 |
| Winter | Pacific herring (*Clupea pallasii*)<br>Plainfin midshipman<br>English sole | 220.6 | 130.8 |

We assume that the spatial overlap between harbor seals and prey species reflects the tendency of harbor seals to frequent areas where the density of prey is greatest. Seasonal correlations between harbor seal locations and prey density revealed the following patterns.

### Spring prey species

The spatial correlation between harbor seals and English sole during spring could reflect harbor seals' use of shallower waters associated with breeding behavior because four of nine of the harbor seals tagged during this season were females of reproductive age, two of which were confirmed breeders (as determined from resightings with a pup). Harbor seal females wean their pups after four weeks and do not fast during the nursing period. Castro Rocks is submerged during high tides, requiring cows and pups to leave the site together. Pups have reduced diving capabilities (Bigg, 1973), which may predispose the females with pups to forage in shallower waters. English sole are abundant in the waters of central SFB and San Pablo Bay during spring, mostly in shoal waters of San Pablo Bay.

The consistent correlations between the distribution of harbor seal locations and crab may be due more to the foraging habits of harbor seal prey than to the harbor seals foraging directly on crab. Harbor seals consume crabs in California (Harvey et al., 1995), but whether crabs are important prey among harbor seals in SFB remains unclear. Pacific staghorn sculpin and English sole are major consumers of juvenile Dungeness crabs, and the vast majority of Dungeness crabs in SFB are juveniles (Reilly, 1983). In addition, crab numbers tend to be greatest in waters around Castro Rocks (particularly during spring), and therefore, the overlap of harbor seals and crab may also be related to proximity to Castro Rocks.

During spring, greatest concentrations of Pacific staghorn sculpin were found in the extreme south bay, indicating that the abundance of this prey species was greater near a large south SFB pupping site, Mowry Slough. These south SFB aggregations of Pacific staghorn sculpin were apparently not used by Castro Rocks harbor seals during spring, and the lack of correlation between harbor seals and sculpin during this season likely reflects the tendency of harbor seals to remain closer to their primary haul-out site during pupping.

### Summer prey species

English sole appear to be an important food source for SFB harbor seals. English sole use the SFB as a nursery area and remain in the bay for 6–18 months (Budd, 1940). Although there was a significant correlation between harbor seal locations and English sole abundances in all seasons (see also spring prey species, above), abundance of age-1+ sole was less in the summer, when the correlation between harbor seals and English sole was greatest. Because harbor seals are opportunistic foragers, they may exploit the abundant young English sole found near their haul-out site during the molting season. Large mature English sole (> 250 mm) were rarely caught by CDFG surveys, and Torok (1994) suggested that any foraging on flatfish by south SFB harbor seals took place outside of SFB; however, harbor seals were occasionally observed feeding on large flatfish near the Castro Rocks site. In addition, harbor seals often visited areas where Pacific staghorn sculpin were numerous, particularly in waters around the Castro Rocks haul-out site. Harbor seals spend a greater proportion of their time hauled out during the molting season (Thompson et al., 1989), and harbor seals in this

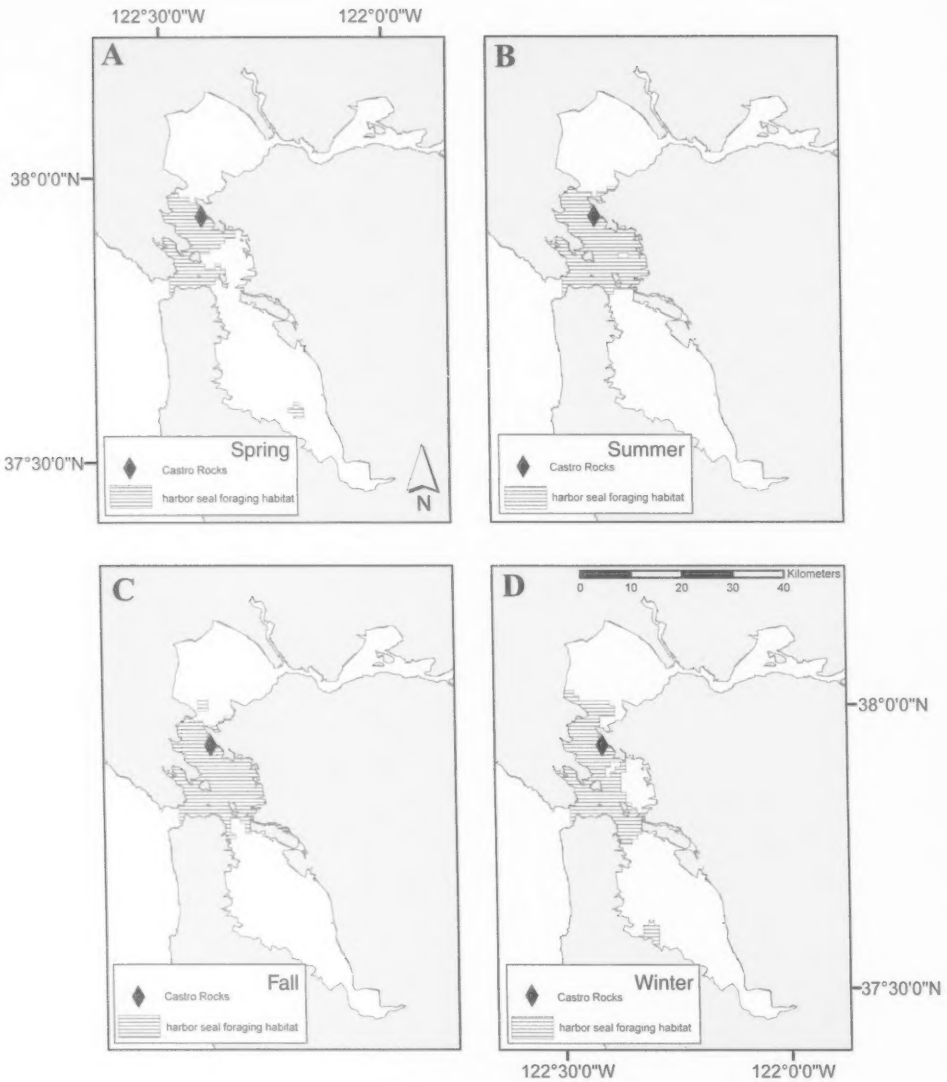


**Figure 5**

Pacific harbor seal (*Phoca vitulina richardii*) foraging habitat in San Francisco Bay, CA, by season: **A**) Spring; **B**) Summer; **C**) Fall; D) Winter. Areas were designated harbor seal habitat if they contained abundances of primary seasonal prey species which met or exceeded the thresholds identified in the regression tree analyses. Primary seasonal prey species were identified based on strength of correlation in the GIS overlay analysis between harbor seal telemetry locations and abundance of individual prey species.

study were likely foraging on these species because of their close proximity to the harbor seals' primary haul-out site during the annual molt. Longer-range foraging trips by harbor seals tend to occur outside of the molting and pupping seasons (Thompson et al., 1989).

### Fall prey species

Harbor seals in SFB frequently visit areas where benthic fish species common in SFB are found in greatest abundance. Adult plainfin midshipman tend to burrow into the sand during the day, emerging at night to

feed (Fitch and Lavenberg, 1971). Harbor seals in SFB forage both during the night and day (Torok, 1994; Nickel, 2003), so harbor seals may be feeding on adult plainfin midshipman at night, in the same areas where greater numbers of juveniles were recorded during the day by CDFG trawls; spatial distribution of adult and juvenile plainfin midshipman is similar in SFB. Numbers of plainfin midshipman are greatest in central SFB during the fall. In addition, white croaker are abundant in SFB; age-1+ white croaker move into central SFB in the fall before migrating out of SFB in the late fall and winter. During fall, harbor seals occurred where there were greatest numbers of this species in central SFB and around Castro Rocks. The Pacific staghorn sculpin is one of the most abundant demersal fish in SFB, and is common in central SFB and San Pablo Bay. Numbers of adults are greatest during October through April. Harbor seals are more frequently located in areas with greatest abundance of this species, in both the fall and summer.

### Winter prey species

The waters to the northeast of the Tiburon Peninsula and near the southeastern edge of Angel Island appear to represent important foraging areas for SFB harbor seals, particularly those using the Castro Rocks haul-out site. These areas were frequently visited by harbor seals year-round, and use of these areas has been noted in earlier studies of harbor seals in SFB (Torok, 1994, Nickel, 2003). During winter, abundance of plainfin midshipman was greatest in central SFB, most notably in waters to the northeast of the Tiburon Peninsula and the southeastern edge of Angel Island. Similarly, maximum numbers of English sole were found in the central bay, around Tiburon Peninsula and Angel Island.

Pacific herring is believed to be a preferred prey of harbor seals in SFB, and seasonal distribution of harbor seals in SFB reflects seasonal increases in abundance of Pacific herring, which spawn in SFB during the winter (Grigg, 2008). Correlations between the distributions of Pacific herring and harbor seals were greatest during winter, and harbor seals often were seen in SFB covered with herring eggs. Well-known Pacific herring spawning areas, such as Richardson Bay, around the Tiburon Peninsula, and along the eastern shoreline of SFB from Castro Rocks southward, were visited frequently by harbor seals during this season.

Harbor seals do not appear to focus much foraging effort on Chinook salmon while they move through SFB. This part of the analysis may have been limited by the very small numbers of salmon caught by CDFG trawls. However, salmon were not reported in earlier studies of harbor seal diet in SFB based on fecal sample analyses (Torok, 1994).

Overall, our findings agree with previously reported harbor seal diets based on fecal sample analyses in SFB (Torok, 1994), with the notable exception of yellowfin goby. Torok (1994) found that yellowfin goby was the most numerous species in harbor seal fecal samples, although his samples were primarily collected from harbor seals captured in the south SFB, whereas the tagged harbor seals used in this study were captured at a central SFB haul-out site. Yellowfin gobies are seasonally abundant in San Pablo Bay and Suisun Bay. Although both areas were visited by harbor seals in our study, these harbor seals may have foraged on gobies opportunistically, because they did not appear to frequently visit areas with abundant yellowfin goby. This may reflect partitioning of foraging habitat between harbor seals using primarily south SFB haul-out sites and using central and north SFB haul-out sites; separation of harbor seals in northern and southern areas of SFB has been noted before (Allen et al., 1993; Grigg, 2008). Use of haul-out specific foraging areas has been noted in other harbor seal populations (Iverson et al., 1997; Thompson et al., 1998).

The accuracy of our methods for identifying foraging habitat for harbor seals in this region is supported by the fact that our findings agree with earlier studies, which demonstrate the tendency of harbor seals to forage on seasonally abundant, primarily benthic prey found near their primary haul-out site (e.g., Harkonen, 1987; Thompson et al., 1998). Spatial overlap between harbor seals and the prey species surveyed in this study was greatest in waters within approximately 10 km of Castro Rocks and declined in waters beyond this range. This is consistent with central place foraging theory, where site use is expected to decline with distance from the central place (e.g., haul-out site; Orians and Pearson, 1979). There may also be a tendency for harbor seals to feed in areas with more predictable prey, e.g., the waters of central SFB, where abundance of fish caught in CDFG trawls tends to be high year-round. Greater correlations between predator and prey can be expected in these areas where prey is predictably present, and lesser correlations in other less predictable, "ephemeral" prey areas (Davoren et al., 2003), e.g., other parts of SFB. Similarly, harbor seals may select a larger-scale region (e.g., central SFB) where prey are found in greater abundance, a trend that is reflected in the greater correlations at larger scales in our study and in other studies of marine predators and their prey (Rose and Leggett, 1990; Mehlum et al., 1999; Fauchald et al., 2000). Harbor seals' tendency to return repeatedly to the same foraging areas indicates that they can track regions with predictably abundant prey over long time scales (e.g., seasonally), despite the likelihood that locations of individual prey patches may vary over much shorter time scales. In other systems and at smaller scales, prey patches are more variable in location, and correlations between predators and prey at smaller scales likely will be weaker (Mehlum et al., 1999). Harbor seals tend to choose haul-out sites located near abundant prey resources (Loughlin, 1978), and the selection of the Castro Rocks location as a haul-out site almost certainly reflects availability and abundance of prey resources nearby.

We did not attempt to identify average patch size of harbor seal prey in our study, choosing instead to demonstrate overlap with areas of abundant prey resources. As Dungan et al. (2002) noted, patches that are smaller than the size of the sampling unit cannot be detected. Average size of prey patches in SFB may be smaller than our minimum study scale of 1 km. Nickel (2003) suggested that the average maximum prey patch for harbor seals foraging in SFB was 200 m, based on fractal analyses of harbor seal positions obtained by VHF radiotracking. If true, our grid cells could potentially have contained no prey patches or more than one prey patch, and could illustrate relative quality of foraging areas rather than locations or sizes of individual prey patches. On the other hand, we would expect positive correlations between harbor seal locations and prey densities only at scales greater than the dimensions of aggregations of predator or prey. At scales less than the smallest aggregation of predator or prey, we would be more likely to see negative correlations, because of factors such as predator avoidance by prey (Rose and Leggett, 1990). The greater correlations seen at larger scales in our study may also reflect the tendency of large scales to reduce or average out the effects of stochastic processes affecting where prey patches are located (Corsi et al., 2001). Small but significant negative correlations were found only at smaller scales (1 to 4 km) in our study, primarily involving two species: jacksmelt (spring and summer) and yellowfin goby (spring). Jacksmelt are often found in large schools in SFB, and negative correlation of the jacksmelt and harbor seal distributions at smaller scales could be due to avoidance of foraging harbor seals by jacksmelt schools. Catch per unit of effort of yellowfin goby is usually greatest in San Pablo Bay and Suisun Bay, and least in central SFB, which could explain the negative correlation with this species.

Based on observations of foraging locations of individual SFB harbor seals seen in past studies (Fancher, 1979; Torok, 1994), the amount of harbor seal habitat estimated by our methods may be conservative. Our estimates were based on only the species with the strongest correlations between harbor seal locations and prey abundance data during our study period. Had additional species' distributions been included in the potential habitat maps, a greater proportion of SFB may have been designated as foraging habitat for harbor seals. In addition, our estimates were based on strong correlations between predator and prey for harbor seals captured at (and presumably using) a haul-out site in central SFB. Had harbor seals used in this study been captured at a site in the extreme southern or northern SFB, the relative strength of correlations between harbor seals and individual prey species may have been different, if harbor seals that use these other sites focus on different prey species. However, both an earlier radiotracking study using harbor seals captured in southern SFB (Torok, 1994) and a 2000–2001 radiotracking study conducted using harbor seals captured at Castro Rocks (Nickel, 2003) identified large foraging areas in central SFB, a number of which fell within the same areas indicated by our habitat maps. In addition, as harbor seals will shift prey species with seasonal and annual changes in local prey abundance (Tollit and Thompson, 1996), significant changes in the abundance or distribution of prey species in SFB could cause harbor seals to switch to foraging areas not identified in our maps. With new information on prey distribution, locations of potential foraging habitat could easily be updated in the GIS.

Identifying factors that govern when harbor seals leave SFB to forage will require larger sample sizes, information on seasonal patterns of prey availability on the outer coast, and possibly more fine-grained data on individual harbor seal movements and behavior while at sea. In many areas, including SFB, harbor seals exhibit two foraging strategies (Thompson et al., 1998; Grigg, 2008). In one strategy, harbor seals make shorter, daily trips to and from foraging areas near the haul-out site; in the alternative strategy, harbor seals make longer foraging trips to more distant foraging areas, often lasting for a number of days and followed by extended haul-out periods. Harbor seals often move to protected estuarine haul-out sites to breed and molt, and numbers of harbor seals at some haul-out sites in SFB are greatest during these seasons (Grigg et al., 2004). The proportion of harbor seal locations on the outer coast in our study was greater during the spring (pupping) and summer (molting) seasons and may reflect the need for some individual harbor seals to forage in coastal waters when the density of harbor seals was high in SFB, in order to minimize intraspecific competition for prey (and therefore, animals would forage in coastal waters but return to SFB haul-out sites to rest between trips). Alternatively, this behavior could reflect the movement by some harbor seals to pupping or molting haul-out sites located outside of SFB, and use of coastal foraging areas closer to these haul-out sites. Inspection of individual harbor seal movements (not shown) indicated that both patterns were probably occurring during our study period (Grigg, 2008).

Satellite telemetry enabled us to identify correlations between harbor seal distribution and the distribution of prey species known to be present in the diet of local harbor seals. Using the harbor seal locations and GIS overlay analyses, we identified species of fish likely to be important seasonal prey species of harbor seals in SFB, and areas likely to be important foraging areas (particularly for harbor seals using the Castro Rocks haul-out site). Use of Service Argos positions required substantial elimination of inaccurate positions, with resultant loss of sample size and resolution of harbor seal movements, and limited the scale at which the analyses could be conducted. Nonetheless, we feel that satellite telemetry provided a useful way to assess harbor seal distribution in our coastal study area, allowing continual collection of information on harbor seal locations that would have been difficult using currently available VHF radiotelemetry tags, and eliminating the potential disturbance of an observer's presence on foraging harbor seals.

Finally, this spatially explicit approach to the identification of foraging areas has applications to management of pinnipeds in other areas, and other marine predators that can be tracked with satellite telemetry. With local data inputs comparable to those used in this study, this approach could be applied to management of marine predators in other areas. Knowledge of the location of foraging areas is important to such management concerns as the potential effects of future shifts in the spatiotemporal distribution of prey (due to climate change, etc.), the link between local resource availability and local population trends, prediction of possible interactions of marine predators with fisheries, or identification of appropriate locations for marine protected areas.

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# Use of pop-up satellite archival tag technology to study postrelease survival of and habitat use by estuarine and coastal fishes: an application to striped bass (*Morone saxatilis*)

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**Abstract**—Pop-up satellite archival tags (PSATs) have been used to study movements, habitat use, and postrelease survival of large pelagic vertebrates, but the size of these tags has historically precluded their use on smaller coastal species. To evaluate a new generation of smaller PSATs for the study of postrelease survival and habitat use of coastal species, we attached Microwave Telemetry, Inc., X-tags to ten striped bass (*Morone saxatilis*) 94–112 cm total length (TL) caught on J hooks and circle hooks during the winter recreational fishery in Virginia. Tags collected temperature and depth information every five minutes and detached from the fish after 30 days. Nine of the ten tags released on schedule and eight transmitted 30% to 96% (mean 78.6%) of the archived data. Three tags were physically recovered during or after the transmission period, allowing retrieval of all archived data. All eight striped bass whose tags transmitted data survived for 30 days after release, including two fish that were hooked deeply with J hooks. The eight fish spent more than 90% of their time at depths less than 10 m and in temperatures of 6–9°C, demonstrated no significant diel differences in depth or temperature utilization ($P>0.05$), and exhibited weak periodicities in vertical movements consistent with daily and tidal cycles.

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Developments in pop-up satellite archival tags (PSATs) have greatly improved scientific understanding of the postrelease survival, behavior, and movements of marine vertebrates—animals from which it is not always practical to physically recover tags to obtain data (Arnold and Dewar, 2001; Graves et. al. 2002). PSATs take measurements of physical conditions (e.g., temperature, pressure, light level) while attached to study animals, independently detach at predetermined times, float to the surface, and transmit data to orbiting satellites of the Argos system. Owing to the mass and size of older tags (~65 g), PSAT deployments have historically been limited to large pelagic marine vertebrates such as billfishes, tunas, sharks, and sea turtles. Recent miniaturization of tag components has led to the development of a new generation of PSATs that are 33% smaller, thus enabling the collection of high-resolution time-series data for inferences regarding short-term fate and habitat use by increasingly smaller species, including many estuarine and coastal fishes.

To evaluate the utility of the new generation of smaller PSATs for studies of estuarine and coastal fishes, we deployed ten tags on large, coastal, migratory striped bass (*Morone saxatilis*) caught on live baits rigged on two hook types in the winter recreational fishery off coastal Virginia and North Carolina. Although smaller PSATs provide opportunities to investigate smaller species, coastal and estuarine fishes and the characteristics of their habitats present special challenges for PSAT deployments. First, many coastal species associate with physical habitat structures in which the tags could become entangled, possibly resulting in premature release of the tag. Secondly, many coastal species aggregate, providing opportunities for conspecifics or other species to interact with the tag, possibly causing premature release or damage to the PSAT. Finally, because coastal species are found near shore, there is an increased probability that a released (transmitting) PSAT will wash ashore during the transmission period, potentially reducing the quality and quantity of subsequent data transmissions. On the other hand, the increased probability of beaching during data transmission may provide researchers opportunities for directed tag recovery.

A second goal of this study was to gain insights into the postrelease survival of striped bass released from recreational fishing gear during the winter prespawning aggregation near the mouth of Chesapeake Bay. Striped bass are a highly prized recreational gamefish, providing over $300 million to the U.S. economy and over $60 million to Virginia annually (Kirkley and Kerstetter, 1997; Richards and Rago, 1999). Management regulations, such as seasonal

**Table 1**

Summary of published postrelease survival experiments using J, treble, and circle hooks conducted on striped bass (*Morone saxatilis*) released from the recreational fishery. F=freshwater, S=saltwater followed by the state abbreviation. Hook types are: J (straight-shank J hook), C (circle hook), and T (treble hook). For release mortality, estimates are for artificial lures (L), live bait (B), J hooks (J), or circle hooks (C).

| Source | Water type and region | Season | Hook | Bait type or lure | Release mortality |
|---|---|---|---|---|---|
| Harell (1988) | F | Winter, summer | J | Live bait, lures | L: 15.6%, B: 30.7% |
| Hysmith et al. (1993) | F: TX | Winter, summer | J | Live bait, lures | 38% |
| Diodati and Richards (1996) | S: MA | Summer | J | Live bait, lures | 3–26%; mean 9% |
| Nelson (1998) | F: NC | Spring | J, T | Live baits, lures | 6–27%: mean 6.3% |
| Bettoli and Osborne (1998) | F: TN | Winter, summer | J , T | Live baits, lures | 14–67% |
| Lukacovic and Uphoff (2002) | S: MD | Summer | J | Natural baits | J: 9.1% |
| | | | C | | C: 0.8% |
| Millard et al. (2003) | F: NY | Spring | J | Natural baits | 8–18% |

bag and size limits, have resulted in the release of over 90% of the striped bass caught by recreational anglers (Van Winkle et al., 1988). Current recreational postrelease mortality estimates for striped bass range between 3% and 67%, and a value of 9% is currently used in population assessments for the Chesapeake Bay stock (Diodati and Richards, 1996). However, previous studies have generally been conducted in fisheries and environmental conditions very different from those near the mouth of Chesapeake Bay during the winter months (Table 1).

A third goal of this study was to determine habitat use by coastal migrant striped bass during the winter prespawn aggregation in the coastal sea along Virginia. Habitat use by juvenile striped bass within estuarine and riverine waters has been fairly well studied (Tupper and Able, 2000; McGrath, 2005), as have the movements of adults during upriver spawning migrations (Carmichael et al., 1998). Little is known about the depth and temperature use or short-term movements of adult striped bass in winter prespawning aggregations along the U.S. Mid-Atlantic coast, despite the importance of Chesapeake Bay to the coastal migrant population. The Chesapeake Bay stock is thought to be the most productive along the Atlantic coast, serving as a major source of coastal recruits and accounting for >90% of Atlantic coastwide landings in some years (Kohlenstein, 1981; Richards and Rago, 1999; Secor, 2000). Identifying habitat characteristics and patterns of habitat use by coastal migrant species in areas of aggregation are necessary for effective current and future management efforts (Carmichael et al., 1998; Conrath and Musick, 2008).

## Materials and methods

The X-tag high rate archival tag (X-tag, Microwave Telemetry, Inc., Columbia, MD) used in this study is slightly buoyant, and weighs 40 g in air. The body of the tag contains a lithium composite battery, a microprocessor, a pressure sensor, a temperature gauge, a light sensor, and a transmitter, all encased within a carbon fiber housing. Flotation is provided by a spherical resin bulb embedded with buoyant glass beads and the tag can withstand pressure equivalent to a depth of 2500 m. This tag model was programmed to record and archive a continuous time series of temperature, light, and pressure (depth) measurements approximately every five minutes for 30 days. The tags can transmit depth measurements at intervals of approximately 1.3 m and temperature in increments of 0.17°C. Not having prior information on the time course or range of vertical movements of striped bass overwintering off the mouth of Chesapeake Bay, we chose not to activate an optional feature that provides for early tag release in the case of a mortality which is inferred if the tag remains at constant depth (±1.5 m) for four days. The X-tags were equipped with Satellite in View™ software that increases battery life and data recovery by restricting transmissions to times during which there is a high likelihood that the Argos satellite will pass above the horizon.

Striking a balance between availability and size of striped bass in the winter recreational fishery off the mouth of Chesapeake Bay, we arbitrarily set a minimum length threshold for tagging of 94 cm total length (TL). Striped bass in this size range are sexually mature coastal migrants (Dorazio et al., 1994) that weigh 8 kg or more (Secor, 2000) and were considered to be of sufficient size to carry the X-tag.

Striped bass were caught by using live eels (*Anguilla rostrata*) as bait on 13.6-kg test sportfishing tackle with 1.2-m leaders of 36.3-kg test line. Five striped bass were caught on J hooks (Gamakatsu Octopus, size 7/0, no offset), and five on circle hooks (Gamakatsu Octopus Circle, size 7/0, no offset). Fish were netted and brought on deck where the hook location was noted, the hook removed, total length measured, and the PSAT attached before the fish was returned to the water (air exposure time less than two minutes).

PSATs were attached to striped bass by an assembly composed of 16 cm of 182-kg test monofilament fishing

**Figure 1**

X-tag (Microwave Telemetry, Inc., Columbia, MD) attached to a striped bass (*Morone saxatilis*). The nylon intramuscular tag anchor was inserted approximately 5 cm towards the dorsal midline, an area where the anchor had a high likelihood of securely interlocking with the pterygiophores supporting the dorsal fin spines.

line (Momoi Fishing Co., Ako City, Japan) attached to a large, hydroscopic, surgical-grade nylon intramuscular tag anchor according to the method of Graves et al. (2002). Attachment assemblies were implanted with a 5-cm stainless steel applicator attached to a 0.3-m tagging pole that was inserted behind a scale approximately 5 cm deep into a target region approximately 6 cm posterior to the origin and 5 cm ventral to the base of the dorsal fin (Fig. 1). In this region, the nylon anchor can pass through and potentially interlock with pterygiophores supporting the dorsal fin well above the coelomic cavity containing visceral organs (Graves et al., 2002).

### Data analyses

Net movement was calculated as a minimum straight line distance (MSLD) traveled between coordinates of initial tagging and coordinates of the first reliable satellite transmission by using Argos location codes 1, 2, or 3 (Horodysky et al., 2007). Archived and transmitted point measurements of depth and temperature recorded by PSATs were summarized in 5-m and 1°C interval histograms. Data sets were truncated to remove records before tagging and after PSAT pop-up.

To assess potential diel differences in habitat utilization, mean depths and temperatures were generated for each diel period (day, night) of each tracking day ($n$=30) for each of the eight striped bass. Diel period designations were based on times of local sunrise and sunset; crepuscular periods (30 minutes on either side of dawn and dusk) were eliminated from all diel analyses. Diel differences in the depth and temperature means were assessed separately with linear mixed effects models of the following form (Pinheiro and Bates, 2004):

$$Y_{pi} = \mu + \tau_p + \alpha_i + \varepsilon_{pi}, \quad (1)$$

where $\mu$ = the overall mean depth or temperature;
$\tau_p$ = the fixed effect of diel period $p$;
$\alpha_i$ = the random effect due to individual fish; and
$\varepsilon_{pi}$ = error terms.

Application of linear models requires satisfying three assumptions: independence and normality of the response within and among samples, and homogeneity of variances among all levels of the fixed effects (Underwood, 2002). However, PSAT data constitute repeated nonindependent observations within individual fish and may fail to satisfy the assumptions of normality and homogeneity of variance. Accordingly, a repeated measures form of Equation 1, including a Box-Cox transformation of the depth and temperature data, rectified these problems in the striped bass data. To characterize the within-individual autocorrelation, several candidate covariance structures were fitted to the transformed depth and temperature data, and the appropriate structure was selected by using Akaike's information criterion ($AIC$):

$$AIC = -2\ln(\hat{L}) + 2p, \quad (2)$$

**Table 2**

Hook type, hooking location, release date, fish size, PSAT data recovery, and net movement data for striped bass (*Morone saxatilis*) caught on live eels (*Anguilla rostrata*) in the winter recreational fishery off the coast of Virginia and North Carolina. Starred (*) data recovery percentages indicate instances where PSATs were physically recovered, allowing a full download of all archived data. Minimum straight line displacements (MSLDs) were calculated in nautical miles (nmi) from the coordinates of tagging to the coordinates of first reliable satellite contact (Argos location code 1, 2, or 3).

| Fish | Hook type | Hooking location | Date released | Total length (cm) | Data recovery (%) | MSLD (nmi) |
|---|---|---|---|---|---|---|
| 1 | J | Deep | 26 Jan 08 | 94.0 | 90 | 29.9 |
| 2 | J | Upper jaw | 26 Jan 08 | 94.0 | 100* | 56.3 |
| 3 | C | Jaw corner | 26 Jan 08 | 96.5 | 87 | 27.8 |
| 4 | C | Upper jaw | 27 Jan 08 | 111.8 | 100* | 34.3 |
| 5 | C | Jaw corner | 27 Jan 08 | 94.0 | 90 | 58.6 |
| 6 | J | Deep | 2 Feb 08 | 96.5 | 96 | 12.5 |
| 7 | C | Upper Jaw | 2 Feb 08 | 104.1 | 30 | 27.1 |
| 8 | J | Upper Jaw | 2 Feb 08 | 101.6 | 100* | 32.5 |

where $\hat{L}$ = the estimated value of the likelihood function at its maximum; and
$p$ = the number of estimated parameters (Burnham and Anderson, 2002).

We performed fast Fourier transform (FFT) analyses to assess any periodicities inherent in the time series of the three recovered tags for which 100% of the archived data were obtained. FFT approximates a function composed of sine and cosine terms from a time series (Chatfield, 1996) and is particularly well suited to analyzing high-resolution data sets resulting from archival tagging studies (Graham et al., 2006; Shepard et al., 2006). The influence of periodic components in a time series is indicated by the magnitude of the corresponding spectral peak in a periodigram (Shepard et al., 2006). Spectral components of fractional periodicities (i.e., part of a tidal cycle, moon phase, etc.) occurring before and after the tag deployment duration can interfere with each other, generating frequency peaks that do not represent meaningful behavioral periodicities (Shepard et al., 2006). We therefore applied a Hamming window to the depth records of each of the three striped bass to reduce the effects of such adjacent spectral components (Oppenheim and Schafer, 1989). All statistical analyses were performed with the software package *R*, vers. 2.7.1 (R Development Core Team, 2008).

## Results

Ten striped bass, ranging in size from 94 to 112 cm TL (mean=96.5 cm), were caught on live eels rigged with circle or J hooks in coastal waters (<20 m depth) of Virginia and North Carolina during late January and early February 2008 (Table 2). Fight times ranged from 1 min 10 sec to 5 min 30 sec (mean=2 min 16 sec). All five fish caught on circle hooks were hooked externally, either in the upper jaw or the corner of the jaw. Two of five fish caught on J hooks were hooked deeply and the other three were hooked externally. Hooks were removed from all fish before they were tagged and released.

Eight of the ten PSATs popped up on schedule and transmitted data that were received by satellites of the Argos system. A single, weak transmission was received from one of the two remaining tags on the day it was scheduled to release, and no transmissions were received from the other PSAT. The tags had sufficient battery power to transmit data for approximately 30 days, and during that time three of the eight reporting PSATs washed ashore. Two of these tags (from fish 2 and 4) were physically recovered while transmitting data. Transmissions from the third tag (fish 7) ceased when the PSAT washed ashore four days after surfacing; this tag was not recovered. A fourth tag (fish 8) remained adrift during its transmission period and subsequently washed ashore north of Cape Hatteras, NC, where it was recovered by a recreational angler.

Data recovery rates varied among the eight transmitting tags. All of the archived data were manually downloaded from the three tags that were recovered after having washed ashore. For the four tags that remained adrift during the transmission period and not subsequently recovered (fish 1, 3, 5 and 6), data recovery rates were high, ranging from 87 to 96%. The PSAT from fish 7 surfaced just off the seaside of the Eastern Shore of Virginia and washed ashore on Parramore Island after four days, at which time transmissions ceased to be received. During the four-day transmission period, 30% of the archived data were recovered from this tag.

From a visual inspection of depth and temperature data we inferred that all eight striped bass with reporting tags, including the two fish that were deeply hooked with J hooks, survived for 30 days after re-

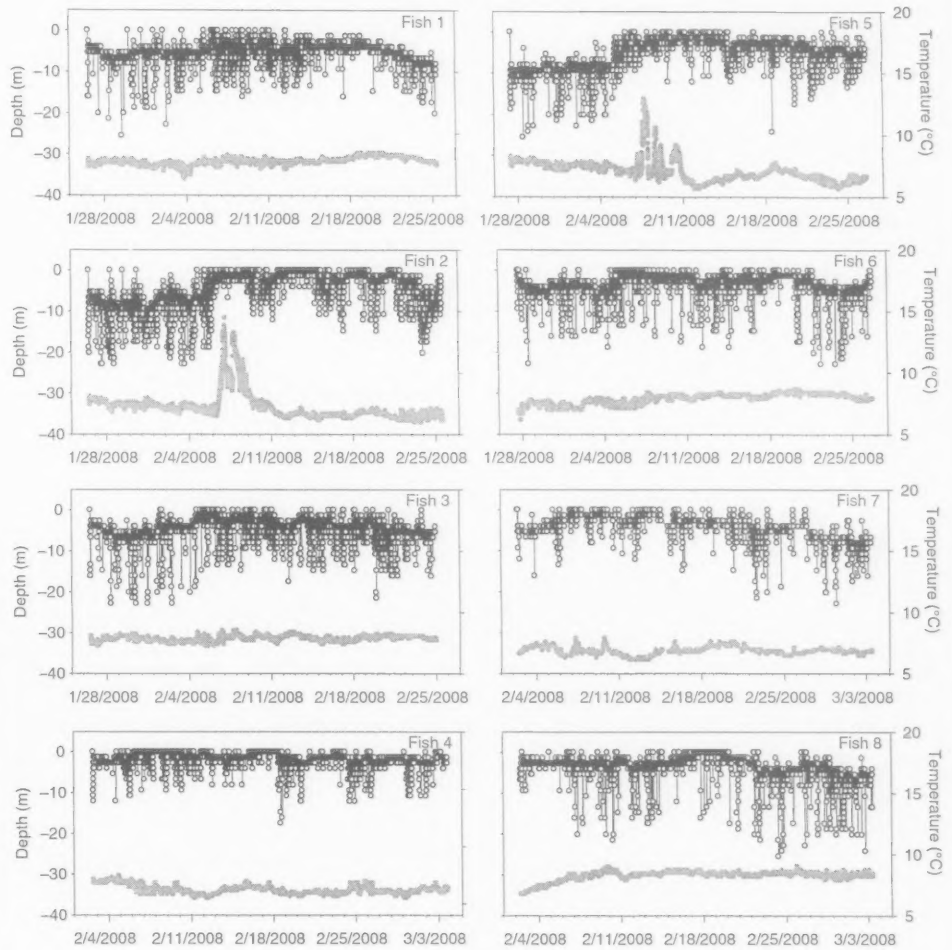


**Figure 2**

Depth (left axis, open black symbols) and temperature (right axis, closed grey symbols) time series from Microwave Telemetry X-tags deployed on eight large coastal migrant striped bass (*Morone saxatilis*) from January through March 2008. Tags for fish 2, 4, and 8 were recovered and represent the full 100% downloaded data streams.

lease. Each fish exhibited multiple vertical movements in the water column throughout the 30-day tagging period (Fig. 2). Inferences of survival based on depth and temperature data were also supported by calculations of net movement (Graves et al., 2002). Minimum straight line displacements for the eight striped bass ranged from 12.6 to 58.6 nautical miles (nmi; 23.3–108.5 km), with a mean of 34.9 nmi (64.6 km; Fig 3). During the 30-day tagging period, three individuals (fish 2, 4, and 5) left coastal waters and entered Chesapeake Bay, presumably initiating spawning migration.

Depth and temperature data archived by the eight transmitting X-tags demonstrated that coastal migrant striped bass spent >90% of their time in the upper 10 m of the water column in temperatures of 6–9°C (Fig 4). Two striped bass (fish 2 and 5) entered warm temperatures (~15°C) at approximately the same time on the same date. These individuals, tagged on different days in North Carolina waters, may have moved eastward to a warm core eddy confirmed by satellite temperature imagery for 7 February 2008 (http://marine.rutgers.edu/cool/sat_data, accessed May 2008). It is also possible that these fish instead moved into shallow

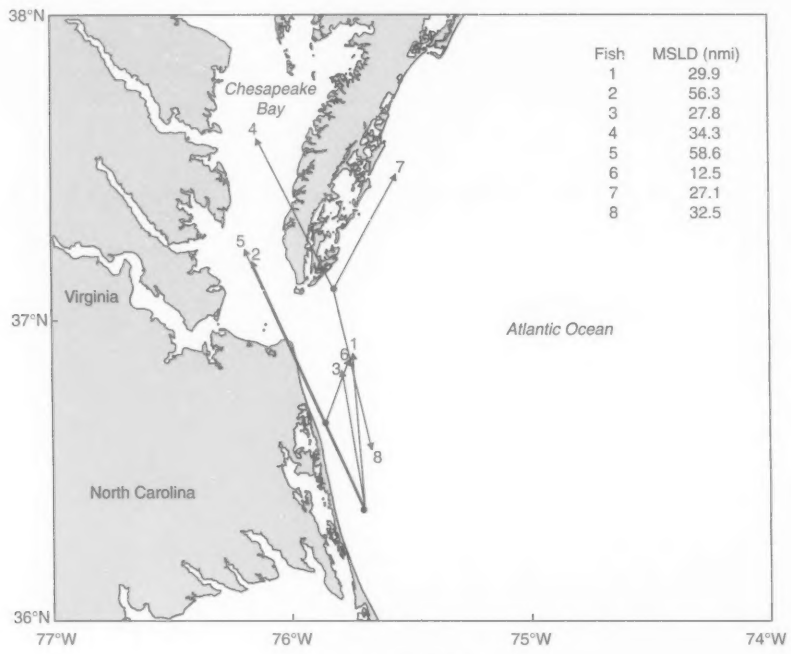


**Figure 3**

Minimum straight line displacements (MSLD) in nautical miles (nmi) of eight large coastal migrant striped bass (*Morone saxatilis*) caught on recreational fishing gear and tagged with Microwave Telemetry X-tags from January through March 2008. Arrow bases (circles) indicate location of fish tagging and release, arrow tips denote the first point of contact with transmitting tag after release from the fish.

coastal or estuarine waters warmed by unseasonable temperatures (~18°C) on 7 February 2008.

Despite the daily variability in the tracks of individuals, repeated-measures linear mixed-effects models yielded no significant diel differences in striped bass depth or temperature utilization ($P>0.05$). The best fitting model for both depth and temperature data was the autoregressive moving average (ARMA) covariance structure.

Fast Fourier transform periodigrams of the three recovered tags revealed weak periodicities in vertical movements consistent with one cycle per day (i.e., 24 hours), and weaker behaviors consistent with two and three cycles per day (i.e., 12 and 8 hours, respectively; Fig. 5). All three periodigrams had large spectral peaks near zero, a consequence of standardizing the depth data by the average depth; main spectral peaks follow this initial clustering (Shepard et al., 2006). The main spectral peaks were identified both with and without the Hamming window, and thus were not attributed to artifact. It is unclear if the periodicities of approximately 12 hours and 8 hours represent specific behavioral cycles or harmonics that result from nonsinusoidal behavior (Chatfield, 1996).

## Discussion

The primary goal of this study was to evaluate the performance of a new generation of smaller PSATs on estuarine and coastal species in the nearshore environment. The larger, older models of PSATs have been deployed on coastal elasmobranchs (Grusha, 2005; Conrath and Musick, 2008). As comparatively smaller coastal and estuarine fishes become candidates for these smaller tags, researchers may wish to consider the minimum size at which drag and lift forces acting on the PSAT impact behavior and survival (Grusha and Patterson, 2005). From the movements of fish and the lack of observed mortalities, we conclude that striped bass of ~1 m TL length appear to be of sufficient size to carry the X-tag.

At the outset of this study we were concerned with the potential for premature release of PSATs because of entanglement in physical structure, fish-tag interactions that would result in premature release or tag damage, and the likelihood that tags would effectively transmit the archived data from nearshore waters. The lack of prematurely released tags in this study confirms that fouling or interactions with structure

were not problematic for striped bass; however, the applicability of these results to other structure-associated species is not known. Premature release of PSATs has been noted in many studies and may become more prevalent with longer deployment times because of attachment methods and increased potential for fish-tag interactions (Domeier et al., 2003; Conrath and Musick, 2008; Graves and Horodysky, 2008). The selection of a specific attachment method and an appropriate release time will depend on the species studied and research objectives of the study (e.g., postrelease mortality, movement, or habitat use).

Fish-tag interactions present challenges for all PSAT studies and may occur as predation of a tag mistaken for a prey item or predation of an individual carrying a tag. Both outcomes are extremely difficult to quantify and compromise study objectives. In schooling piscivorous fishes, such as adult striped bass, predation of PSATs is more likely than predation of the study individuals. We cannot discount that our nonreporting and weakly transmitting tags may have been victims of tag predation; it is often impossible to discern between tag predation and tag failure. However, it is unlikely that mortality of a tagged striped bass would result in a nonreporting tag because the PSAT should surface from a dead carcass after 30 days. The predation of live individuals by elasmobranchs, as well as the scavenging of dead fish carrying PSATs by elasmobranchs, was inferred in previous studies (Kerstetter et al., 2004; Kerstetter and Graves, 2008). In these instances, the PSATs were not compromised during ingestion and successfully transmitted after being regurgitated, but it is likely that damage during such events may be a cause of PSAT nonreportings.

The success of studies where PSAT technology is used depends upon on the quality and quantity of the archived data that are transmitted from the tag to the Argos satellite system. Reception of PSAT transmissions is maximized when the tag antenna is unobstructed and above the surface of the water in a vertical position. In our study, we obtained at least 87% of the data from tags that remained adrift for the entire data transmission period. There is an increased probability that tags attached to estuarine and coastal fishes will wash ashore during the transmission period that typically lasts about 30 days. Tags beach in a horizontal position which may result in decreased signal reception, especially if antennae are submerged in water or fouled with algae or other debris.[1] Beached tags in this study transmitted 30–90% of their data. In the case of the tag attached to fish 7, which beached after only four days of transmission and ceased communicating with the satellite shortly thereafter, the transmission of over 3000 data points provided more than sufficient information to infer survival and investigate habitat use by that individual. The random transmission of data packets (nine consecutive time points) by the X-tags during times when a satellite of the Argos system is likely above the horizon generally results in a rapid accumulation of data during the first week of the thirty-day transmission period (Fig. 6).

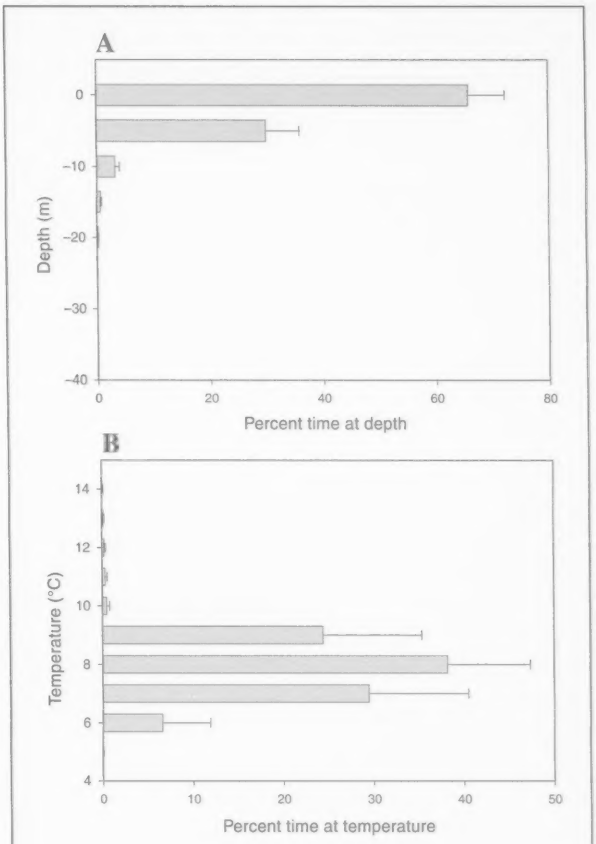


**Figure 4**

Time-at-depth (A) and time-at-temperature (B) histograms from Microwave Telemetry X-tags deployed on eight large coastal migrant striped bass (*Morone saxatilis*) from January through March 2008. Each fish was given equal contribution. Error bars are ±1 standard error.

The two tags that were recovered while still transmitting (fish 2, 4) were carried by fish that moved from coastal waters into the mainstem of Chesapeake Bay. We timed the X-tags to release while striped bass were in coastal or estuarine waters before their annual spring spawning migration to freshwater. The release mechanism on the PSAT, which operates by electrolysis, requires a >5 ppt salinity to function,[1] which necessi-

[1] P. Howey. 2009. Personal commun. Microwave Telemetry, Inc., 8835 Columbia 100 Parkway, Suites K & L, Columbia, MD 21045

tates consideration when dealing with anadromous or catadromous fishes.

PSAT deployments in estuarine and coastal waters will likely have higher tag-to-human interaction rates than those deployed in oceanic waters, and will potentially lead to greater rates of tag recovery. However, to realize these potential benefits, which may be considerable in highly populated regions, the incentive (financial, material, or otherwise) for returning a recovered tag must be sufficient (Pollock et al., 2001). Historically, tag-recovery rates in PSAT studies have been very low. However, Kerstetter and Graves (2008) recently reported recoveries of 4 of 17 PSATs (23.5%) attached to sailfish released from pelagic longline operations in the Gulf of Mexico, south of Key West, FL, and all recoveries came from the heavily used beaches of southeast Florida. Recovery of PSATs can further be aided by the use of radio antennae if tags are transmitting[1]; tags in dense cover can also be located by a metal detector at close range (<0.5 m: A. Horodysky, personal obs.). Tag recovery is beneficial not only because it is possible to obtain 100% of the archived data from the PSAT, but recovered tags can be refurbished for approximately 20% of the cost of a new tag.

A second objective of this study was to assess potential differences in postrelease survival of striped bass caught on live eels rigged with J hooks and circle hooks in the winter recreational fishery. Although the limited sample size precluded statistical comparisons, tags from all eight fish returned data that indicated survival. Circle hooks reduce deep-hooking, hook-induced trauma, and mortality of many fishes (Cooke and Suski, 2004; Horodysky and Graves, 2005), including that of striped bass (Table 1). Previous research has demonstrated a high mortality of striped bass deep-hooked with J hooks and additional and interactive stress-related mortality of larger striped bass caught in warm, low-salinity waters (>20°C, <10 ppt) and handled in still higher air temperatures (>30°C) (Wilde et al., 2000; Lukacovic and Uphoff, 2002). Handling exhausted fish in warmer air can further raise basal metabolic rate, exacerbating oxygen demand and blood chemistry problems (Gingerich, et al., 2007) while simultaneously reducing the gill surface area because of the physical collapse of the gill lamellae and adhesion of the gill filaments (Cooke et al., 2002). We observed 100% survival of tagged fish, including two animals deeply hooked with J hooks, caught in cool, high salinity waters (<10°C, >25 ppt), and handled briefly (<2 minutes) in cool air temperatures (<18°C). Although further work is still needed, the results of these studies indicate that the winter recreational fishery in Virginia may not be a significant source of postrelease mortality for striped bass and that release mortality of this species likely varies temporally and spatially because of physiological stressors.

A third objective of this study was to gain insights into habitat use by striped bass overwintering near the mouth of Chesapeake Bay. Net displacements of the eight fish over the 30-day tagging period were limited, averaging less than 35 nmi (64.8 km). We did not use geolocation algorithms based on light and sea surface temperature data to infer horizontal movements of fish within the 30-day tagging period because the mean displacements over the 30 days were substantially less than the root mean square (RMS) errors associated with daily estimates of geolocation. Under optimal condition, such as

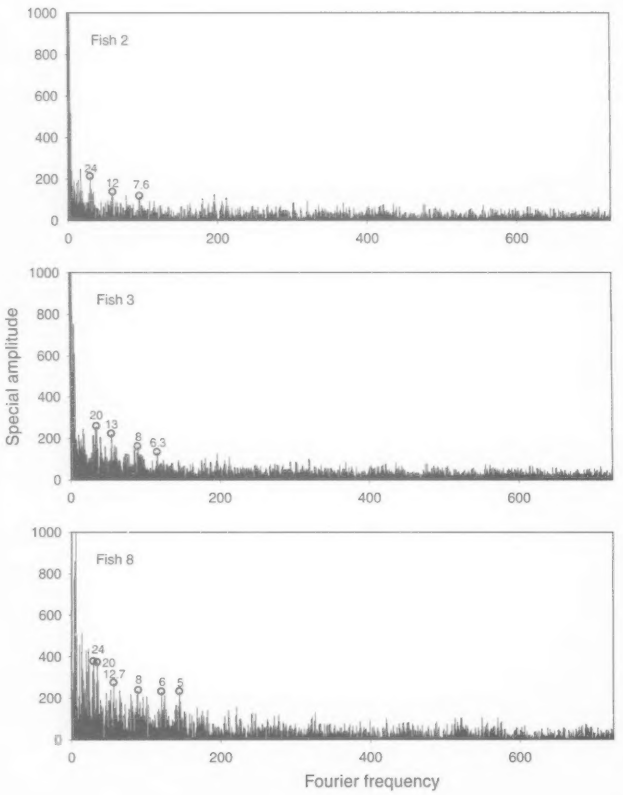


**Figure 5**

Fast Fourier transform periodigrams for depth data from three recovered Microwave Telemetry X-tags (fish 2, 4, and 8) deployed on eight large coastal migrant striped bass (*Morone saxatilis*) from January through March 2008 and physically recovered. Periods of the main spectral peaks found with the raw data and the Hamming window are identified with open circles and labeled in hours.

clear pelagic seas, RMS errors associated with geolocation estimates based on light and sea surface temperature data exceed 100 km (Teo et al., 2004; Nielsen and Sibert, 2007), and the hyperdynamic light conditions characteristic of turbid, tidal coastal waters such as those of Chesapeake Bay, which impede the accurate characterization of sunrise and sunset, would result in even greater RMS errors. Consequently, light-based geolocation would seem to have limited applicability to short-term PSAT studies of estuarine and coastal fishes.

Habitat-use studies based on PSAT data may benefit from analytical frameworks that incorporate repeated measures to account for the inherent within-individual autocorrelation (James et al., 2006; McMahon et al., 2007). Diel differences were not evident in depth or temperature use by coastal migrant striped bass during the January–March tag deployment period. Similarly, there were no significant differences in depth and temperature use among individuals or deployment days. During winter, the adult striped bass staging in coastal Virginia and North Carolina waters forage heavily on dense schools of Atlantic menhaden (*Brevoortia tyrannus*) before traveling into tributaries to spawn (Raney, 1952). The coastal waters of Virginia and North Carolina are fairly shallow and well-mixed, thus the movements of schooling striped bass during our tag deployment duration likely reflect pursuit of prey by a school of predators rather than the selection of preferred depth or temperature ranges by individual striped bass.

Behavioral rhythms in time-series resulting from ultrasonic telemetry and, more recently, recovered PSATs, are ideally analyzed by fast Fourier methods if all data are recovered (Hartill et al., 2003; Shepard, et al., 2006). Fast Fourier analysis of full depth time-series data streams from three recovered PSATs deployed on striped bass indicate subtle daily, 12-hour, and 8-hour periodicities. Daily periodicities may represent onshore-offshore movements of striped bass schools into shallower and deeper waters when they chase menhaden prey, 12-hour periodicities may correspond to ambient diel light regimes, and 8-hour periodicities may indicate subtle tidal or current effects in the use of depth by striped bass. Mid-Atlantic coastal waters and estuaries such as Chesapeake Bay feature semidiurnal tides; tidal stage had substantial impact on movements and habitat use of striped bass in Delaware Bay (Tupper and Able, 2000). Alternately, the 8-and 12-hour periodicities observed in the striped bass data may result from a combination of harmonics resulting from behaviors not strictly sinusoidal in character (Chatfield, 1996). Fourier methods should be applied only to full (100%) data streams to avoid inferring direct spectral relationships between two adjacent data packets that are in reality separated in time by sections of untransmitted archived data.

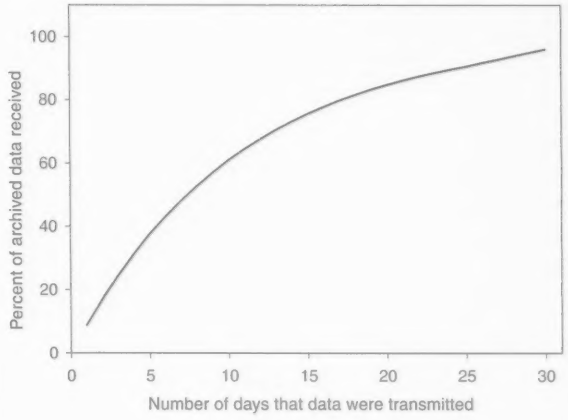


**Figure 6**

Cumulative percentage of archived data that are successfully received by the user as a function of the number of days of transmitted data during the 30-d transmission period for the X-tag high-rate archival tags (Microwave Telemetry, Inc.) programmed with Satellite-In-View (SIV™) technology at Mid-Atlantic latitudes (available from R. P. Howey, 2009, University of Bath, Bath BA2 7AV, UK). Because of the frequency of Argos satellite passes, tags transmitting at higher latitudes will approach asymptotic data recovery more rapidly, and those transmitting at lower latitudes will approach asymptotic data recovery more slowly.

We investigated the applicability of a new generation of smaller PSATs for studies of estuarine and coastal fishes and have provided insights into postrelease survival and habitat use of prespawn aggregating adult striped bass in the winter recreational fishery along the coast of Virginia. Results of this study indicate that tag fouling with physical structures, tag damage resulting from interaction with conspecifics, predators, or scavengers, and reduced transmission efficiency due to beaching or entanglement are not major liabilities for striped bass. In fact, the potential for reduced transmission efficiency is more than offset by increased probability of tag recovery resulting in complete data retrieval and the opportunity to reuse the tag at a greatly reduced cost. Collectively, the results of this study on striped bass indicate that the new generation of smaller PSATs may prove to be an effective tool for studying the postrelease survival of and habitat use by other estuarine and coastal fishes.

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# Food habits of Atlantic white-sided dolphins (*Lagenorhynchus acutus*) off the coast of New England

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**Abstract**—Although the Atlantic white-sided dolphin (*Lagenorhynchus acutus*) is one of the most common dolphins off New England, little has been documented about its diet in the western North Atlantic Ocean. Current federal protection of marine mammals limits the supply of animals for investigation to those incidentally caught in the nets of commercial fishermen with observers aboard. Stomachs of 62 *L. acutus* were examined; of these 62 individuals, 28 of them were caught by net and 34 were animals stranded on Cape Cod. Most of the net-caught *L. acutus* were from the deeper waters of the Gulf of Maine. A single stomach was from the continental slope south of Georges Bank. At least twenty-six fish species and three cephalopod species were eaten. The predominant prey were silver hake (*Merluccius bilinearis*), spoonarm octopus (*Bathypolypus bairdii*), and haddock (*Melanogrammus aeglefinus*). The stomach from a net-caught *L. acutus* on the continental slope contained 7750 otoliths of the Madeira lanternfish (*Ceratoscopelus maderensis*). Sand lances (*Ammodytes* spp.) were the most abundant (541 otoliths) species in the stomachs of stranded *L. acutus*. Seasonal variation in diet was indicated; pelagic Atlantic herring (*Clupea harengus*) was the most important prey in summer, but was rare in winter. The average length of fish prey was approximately 200 mm, and the average mantle length of cephalopod prey was approximately 50 mm.

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The Atlantic white-sided dolphin (*Lagenorhynchus acutus*) is restricted to the temperate and subpolar North Atlantic Ocean, ranging from west Greenland (approximately 64°N) to North Carolina (about 35°N) in the western North Atlantic, and from Norway to the Bay of Biscay in the eastern North Atlantic (Leopold and Couperus, 1995). There are thought to be three populations in the western North Atlantic—the Gulf of Maine, Gulf of St. Lawrence, and Labrador Sea populations (Palka et al., 1997). Seasonal shifts in the Gulf of Maine population have been reported; highest numbers are found in summer and fall and lowest numbers in winter (Northridge et al., 1997; Palka et al., 1997). Most of our animals, however, were obtained during the winter.

This study is the first detailed analysis of the food habits of the Atlantic white-sided dolphin in the western North Atlantic Ocean, hereafter referred to as *L. acutus* rather than the more cumbersome Atlantic white-sided dolphin. Previously, only three live-caught specimens from off New England have been examined for stomach contents and reported in the literature. A single specimen collected in 1954 (Schevill, 1956) and a second collected in 1976, likely a discarded incidental take from a gillnet set near Jeffreys Ledge (Katona et al., 1978), were taken in summer and contained the same species of fish and squid. These records indicated that Atlantic herring (*Clupea harengus*), silver hake (*Merluccius bilinearis*), and northern shortfin squid (*Illex illecebrosus*) could be significant components of the diet. A single animal driven ashore in Trinity Bay, Newfoundland, also contained Atlantic herring and northern shortfin squid (Sergeant and Fisher, 1957).

Other observations have reported a few more prey species for *L. acutus*. Stomachs of 14 of 40 stranded *L. acutus* examined from Cobscook Bay, Maine, contained one silver hake, nine shortfin squid, five rainbow smelt (*Osmerus mordax*), and fragments of unidentified decapod shrimp (St. Aubin and Geraci, 1979; Sergeant et al., 1980). Katona et al. (1978) reported probable feeding on sand lances (*Ammodytes* spp.) by *L. acutus* associated with feeding humpback (*Megaptera novaeangliae*) and fin whales (*Balaenoptera physalus*). The only *L. acutus* feeding incident recorded was on December 20, 1997, on Stellwagen Bank, southwestern Gulf of Maine, when a

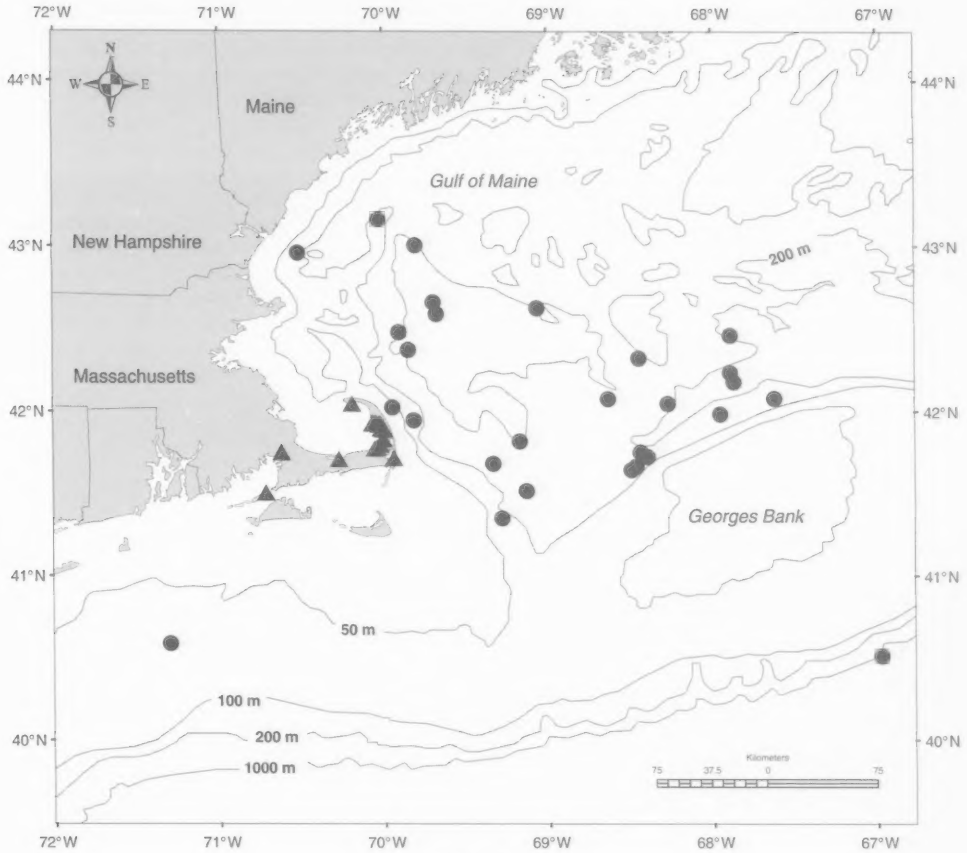


**Figure 1**

Occurrences from 1991 to 2006 of 62 Atlantic white-sided dolphins (*Lagenorhynchus acutus*) collected in the Gulf of Maine and south of Georges Bank and examined for stomach contents in this study. Circles are locations of net-caught dolphins; triangles are locations of stranded dolphins.

group was observed circling and feeding on a school of sand lance (Weinrich et al., 2001).

## Materials and methods

This study records stomach contents from 28 incidentally caught and 34 stranded *L. acutus* from the Gulf of Maine population off the coast of New England (Fig. 1). The animals examined were collected between 1991 and 2006, most of them from 2004–5. The incidentally caught (hereafter referred to as net-caught) animals were taken either by otter trawl (22 stomachs) or by sink gillnet (six stomachs). Of these, 26 were from the Gulf of Maine, one was from deep water (500 m) on the continental slope south of Georges Bank near Munsen Canyon, and one was from the continental shelf south of Narragansett Bay. Thirty-four stomachs were from strandings: 33 from outer Cape Cod, Barnstable County, MA, and one from Naushon, Elizabeth Islands, Dukes County, MA (Table 1).

We have examined every available stomach of net-caught *L. acutus*. All were taken in the U.S. commercial fishery and sampled aboard ship by fisheries observers of the National Marine Fisheries Service (NMFS), Northeast Fisheries Science Center (NEFSC, Woods Hole, MA), Northeast Fisheries Observer Program (NEFOP). NEFOP is the permitting and monitoring agency of the commercial fishing fleet in compliance with the Marine Mammal Protection Act (MMPA) and the U.S. Endangered Species Act (ESA).

Stranded animals were obtained by volunteers of the Cape Cod Stranding Network, currently known as International Fund for Animal Welfare (IFAW), Yarmouth, MA. Most of the stomachs were initially frozen and curated by the NEFSC.

Contents from stomachs determined to be intact (reliable) were examined for frequency, relative abundance,

**Table 1**

Summary comparison of numbers, sex, and stomach contents of 34 stranded vs. 28 net-caught Atlantic white-sided dolphins (*Lagenorhynchus acutus*) collected in the western North Atlantic Ocean off the coast of New England, between 1991 and 2006; and seasonality of the net-caught individuals. Total net-caught includes two calves with empty stomachs; seasonal net-caught excludes the two calves; S. of Georges = South of Georges Bank. Nontrace = whole prey.

| | | | Seasonal net-caught | | |
|---|---|---|---|---|---|
| | Strandings | Total net-caught | Winter | Summer | S. of Georges |
| Number of dolphins | 34 | 28 | 22 | 3 | 1 |
| Depth where captured (m), mean (range) | 0 | 189.8 (55–503) | 186.9 (71–265) | 107.0 (55–187) | 503 |
| Number of males | 22 | 9 | 9 | 0 | 0 |
| Length of males (cm), mean (range) | 227 (156–280) | 208 (173–260) | 208 (173–260) | 0 | 0 |
| Adult males (>230cm) | 10 | 2 | 2 | 0 | 0 |
| Number of females | 12 | 16 | 12 | 3 | 1 |
| Length of females (cm), mean (range) | 205 (168–219) | 199 (161–253) | 203 (171–253) | 178 (161–203) | 252 |
| Adult females (>200cm) | 10 | 6 | 4 | 1 | 1 |
| Number unsexed | 0 | 3 | 1 | | |
| Number of stomachs containing food | 22 (65%) | 25 (89%) | 20 (91%) | 3 (100%) | 1 (100%) |
| Number of empty stomachs | 12 (35%) | 3 (11%) | 2 (9%) | 0 | 0 |
| Number of otoliths | 856 | 10287 | 2157 | 287 | 7843 |
| unidentified | 231 | 54 | 51 | 2 | 1 |
| Number of fish species | 8 | 21 | 17 | 6 | 7 |
| Number of cephalopod beaks | 34 | 535 | 519 | 11 | 5 |
| Number of cephalopod species | 3 | 3 | 3 | 2 | 1 |
| Number of identified prey | | | | | |
| Nontrace fishes | 0 | 50 | 35 | 15 | 0 |
| Near nontrace fishes (skulls) | 7 | 56 | 38 | 4 | 14 |
| Total fishes | 332 | 5179 | 1120 | 135 | 3924 |
| Nontrace cephalopods | 0 | 2 | 0 | 2 | 0 |
| Near nontrace cephalopods | 0 | 1 | 0 | 1 | 0 |
| Total cephalopods | 20 | 282 | 274 | 4 | 4 |

and size (length, weight) of prey. Two net-caught dolphins examined and subsequently eliminated from the analysis were one partly decomposed female with a mud-filled stomach and thoracic cavity and virtually no food in the stomach, and a partially decomposed male with an almost empty stomach taken on the continental shelf south of Narragansett Bay.

Stomachs were weighed whole, divided into their three components (forestomach, main, and pyloric), emptied of contents, and weighed again to determine both the size of the stomach and the mass of its contents. Whole prey (termed "nontrace" prey because they were found relatively intact [not in traces]) were separated, identified, weighed, and measured directly (standard length for fishes and mantle length for cephalopods); well-digested prey (termed "trace prey" because they were found in traces as hard parts, e.g. skull bones, otoliths, jaws, prootic bones of clupeids, teeth and opercula of bony fishes, toothplates of hagfish, and cephalopod beaks and pens) were separated, identified, weighed, and original length measurements were estimated indirectly (standard length for fishes and mantle length for cephalopods). Otoliths were removed from skulls of both nontrace and trace fishes and then cleaned, dried, and measured. The remaining contents were soaked overnight in hot water, if necessary, or elutriated directly through a series of sieves to remove soft tissue and retain hard parts. All items found were identified to the lowest taxonomic level possible (usually to species) by using our own reference collection and published guides (Campana, 2004; Clarke, 1962, 1986; Collette and Klein-MacPhee, 2002; Gregory, 1933; Harkönen, 1986; Smale et al., 1995; Vecchione, 2002). Forestomach contents were then counted and measured and used in all further analyses (Table 2). All fish hard parts, including bones and otoliths, and hagfish toothplates, were stored dried. Cephalopod beaks were removed from buccal masses, cleaned, and stored in 70% ethanol. Crustacean remains and all parasites were counted and also stored in 70% ethanol.

Prey lengths and weights were calculated from otolith lengths for fishes, from lower rostral lengths for squids, and from lower hood lengths for octopods (Tables 3 and 4). Calculations of prey size were used to construct the

**Table 2**

Summary comparison of abundance of identified prey found in the stomachs of both net-caught and stranded Atlantic white-sided dolphins (*Lagenorhynchus acutus*) collected between 1991 and 2006, in the western North Atlantic Ocean off the coast of New England. *n* = number of stomachs; S. of Georges = South of Georges Bank.

| Prey species | Common name | Family | Net-caught winter *n*=20 | Net-caught summer *n*=3 | Net-caught calves *n*=2 | Net-caught S. of Georges *n*=1 | Stranded winter *n*=29 |
|---|---|---|---|---|---|---|---|
| *Alosa pseudoharengus* | alewife | Clupeidae | 2 | | | | |
| *Ammodytes* spp. | sand lance | Ammodytidae | | | | | 271 |
| *Arctozenus risso* | white barracudina | Paralepididae | | | | 5 | |
| *Benthosema glaciale* | glacier lanternfish | Myctophidae | 7 | | | 3 | |
| *Ceratoscopelus maderensis* | Madeira lantern fish | Myctophidae | 1 | | | 3875 | |
| *Clupea harengus* | Atlantic herring | Clupeidae | 5 | 29 | | | |
| *Diaphus dumerilii* | Dumeril's lanternfish | Myctophidae | 1 | | | | |
| *Diaphus mollis* | soft lanternfish | Myctophidae | | | | 2 | |
| *Enchelyopus cimbrius* | fourbeard rockling | Phycidae | 18 | | | | |
| *Gadus morhua* | Atlantic cod | Gadidae | 1 | 4 | | | |
| *Hippoglossoides platessoides* | American plaice | Pleuronectidae | 4 | | | | |
| *Limanda ferruginea* | yellowtail flounder | Pleuronectidae | | | | | 3 |
| *Maurolicus weitzmani* | Atlantic pearlside | Sternoptychidae | 2 | | | | |
| *Melanogrammus aeglefinus* | haddock | Gadidae | 103 | | | | 4 |
| *Merluccius bilinearis* | silver hake | Merlucciidae | 823 | 99 | | 37 | 18 |
| *Myxine glutinosa* | Atlantic hagfish | Myxinidae | 87 | 2 | | | 16 |
| *Nezumia bairdii* | marlin-spike | Macrouridae | 4 | | | | |
| *Osmerus mordax* | rainbow smelt | Osmeridae | | | | | 10 |
| *Scomber scombrus* | Atlantic mackerel | Scombridae | 2 | | | | |
| *Sebastes fasciatus* | Acadian redfish | Scorpaenidae | | 3 | | | |
| *Stomias boa* | boa dragonfish | Stomiidae | | | | 1 | |
| *Tautogolabrus adspersus* | cunner | Labridae | | | | | 9 |
| *Ulvaria subbifurcata* | radiated shanny | Stichaeidae | | | | | 1 |
| *Urophycis chuss* | red hake | Phycidae | 90 | 13 | | | |
| *Urophycis tenuis* | white hake | Phycidae | 4 | | | 1 | |
| *Zoarces americanus* | ocean pout | Zoarcidae | 1 | | | | |
| *Bathypolypus bairdii* | spoonarm octopus | Octopodidae | 210 | | | | 2 |
| *Illex illecebrosus* | northern shortfin squid | Ommastrephidae | 1 | 5 | | | 2 |
| *Loligo pealeii* | longfin inshore squid | Loliginidae | 63 | 1 | | 4 | 16 |
| Total | | | 1429 | 156 | 0 | 3928 | 352 |

**Table 3**

Analysis of prey in 20 Atlantic white-sided dolphins (*Lagenorhynchus acutus*) incidentally net-caught during winter in the Gulf of Maine between 2001 and 2006. NA = not applicable; * = *Myxine glutinosa* double toothplates, 4 per individual; ** = may include a few *Urophycis tenuis*.

| | Prey species | | | Otoliths or beaks | | | Prey length | | Prey weight | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fishes and cephalopods | Number of occurrences | Number of items | Number of individuals | Number measured | Average length (mm) | Length range (mm) | Average length (mm) | Length range weight (g) | Average (mm) | Weight range (g) | Total weight (kg) |
| *Alosa pseudoharengus* | 2 | 4 | 2 | 4 | 3.7 | 2.5–4.9 | | | | | 0.27 |
| *Benthosema glaciale* | 3 | 13 | 7 | 7 | 0.9 | 0.7–1.4 | | | | | <0.01 |
| *Ceratoscopelus maderensis* | 1 | 1 | 1 | 1 | 2.3 | 2.3 | | | | | <0.01 |
| *Clupea harengus* | 4 | 8 | 5 | 5 | 3.3 | 1.8–4.4 | 202.4 | 97.1–277.1 | 88.2 | 7.4–177.8 | 0.44 |
| *Diaphus dumerilii* | 2 | 2 | 1 | 2 | 2.8 | 2.6–2.9 | | | | | <0.01 |
| *Enchelyopus cimbrius* | 4 | 29 | 18 | 29 | 3.2 | 1.9–4.0 | 148.0 | 48.9–213.6 | 22.2 | 0.9–62.3 | 0.40 |
| *Gadus morhua* | 1 | 2 | 1 | 2 | 11.0 | 11.0 | | | | | 0.32 |
| *Hippoglossoides platessoides* | 2 | 7 | 4 | 7 | 3.1 | 2.5–3.7 | | | | | 0.35 |
| *Maurolicus weitzmani* | 2 | 2 | 2 | 2 | 1.6 | 1.5–1.8 | | | | | <0.01 |
| *Melanogrammus aeglefinus* | 7 | 184 | 103 | 171 | 10.2 | 6.0–13.8 | 203.2 | 87.4–327.2 | 91.7 | 5.9–341.2 | 9.44 |
| *Merluccius bilinearis* | 18 | 1646 | 823 | 306 | 9.9 | 3.0–22.4 | 202.5 | 58.6–464.1 | 57.2 | 1.0–669.7 | 27.91 |
| *Myxine glutinosa* | 10 | 332* | 87 | NA | | | NA | | NA | | NA |
| *Nezumia bairdii* | 1 | 6 | 4 | 6 | 5.6 | 4.0–6.2 | | | | | NA |
| *Scomber scombrus* | 1 | 2 | 2 | 2 | 3.8 | 2.5–5.0 | | | | | 0.58 |
| *Urophycis chuss*** | 13 | 191 | 90 | 111 | 8.9 | 1.6–19.1 | 208.9 | 8.5–489.1 | 103.8 | 0.1–809.4 | 11.50 |
| *Urophycis tenuis* | 3 | 7 | 4 | 7 | 14.3 | 12.6–15.6 | | | | | 0.89 |
| *Zoarces americanus* | 1 | 2 | 1 | 2 | 3.0 | 3.0 | | | | | <0.01 |
| Total otoliths | | 2106 | | 664 | | | | | | | |
| Total fishes | | | 1155 | | | | | | | | 52.10 |
| *Bathypolypus bairdii* | 13 | 390 | 210 | 96 | 2.8 | 1.7–4.0 | 49.8 | 36.3–64.5 | 42.6 | 11.2–99.0 | 8.78 |
| *Illex illecebrosus* | 1 | 2 | 1 | 1 | 1.5 | 1.5 | | | | | 0.01 |
| *Loligo pealeii* | 12 | 127 | 63 | 63 | 0.9 | 0.4–1.7 | 51.6 | 16.8–118.9 | 10.9 | 0.8 – 52.8 | 0.69 |
| Total cephalopod beaks | | 519 | | | | | | | | | |
| Total cephalopods | | | 274 | 160 | | | | | | | 9.48 |
| Total all prey | | | 1429 | 824 | | | | | | | 61.58 |

**Table 4**

Analysis of prey in 33 Atlantic white-sided dolphins (*Lagenorhynchus acutus*): 3 net-caught in the Gulf of Maine in summer between 1992 and 2003; 1 net-caught south of Georges Bank in summer 1993; and 29 stranded on Cape Cod in winter between 1994 and 2006. NA = not applicable; * = *Myxine glutinosa* double toothplates, 4 per individual.

| | Prey species | | | Otoliths or beaks (mm) | | | Prey length(mm) | | Prey weight | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fishes and cephalopods | Occurrences | Items | Individuals | Measured | Mean length | Length range | Mean length | Length range | Mean weight (g) | Weight range (g) | Total weight (kg) |
| Net-caught summer Gulf of Maine (3 stomachs) | | | | | | | | | | | |
| *Clupea harengus* | 3 | 56 | 29 | 43 | 4.1 | 3.0–5.0 | 259.7 | 180.2–318.7 | 154.4 | 48.2–271.5 | 3.85 |
| *Gadus morhua* | 2 | 8 | 4 | 8 | 4.5 | 4.3–5.0 | 76.0 | 67.2–89.2 | 5.0 | 3.29–7.55 | 0.03 |
| *Merluccius bilinearis* | 2 | 193 | 99 | 79 | 6.9 | 2.6–12.0 | 139.8 | 50.2–246.7 | 18.0 | 0.6–91.5 | 1.78 |
| *Myxine glutinosa* | 1 | 7* | 2 | NA | | | | | | | |
| *Sebastes fasciatus* | 1 | 4 | 3 | 4 | 1.7 | 1.1–2.1 | | | | | |
| *Urophycis chuss* | 1 | 24 | 13 | 23 | 2.2 | 1.5–4.7 | 25.0 | 5.7–93.6 | 0.4 | 0.05–4.1 | <0.01 |
| *Illex illecebrosus* | 2 | 9 | 5 | 5 | 2.8 | 1.8–3.5 | 145.2 | 96.3–179.4 | 70.9 | 18.3–115.9 | 0.35 |
| *Loligo pealeii* | 1 | 2 | 1 | 1 | 1.5 | 1.5 | 117.2 | | 40.5 | | 0.04 |
| Total | | 303 | 156 | | | | | | | | 6.05 |
| Net-caught summer south of Georges Bank (1 stomach) | | | | | | | | | | | |
| *Arctozenus risso* | 1 | 9 | 5 | 6 | 3.4 | 3.3–3.6 | | | | | |
| *Benthosema glaciale* | 1 | 5 | 3 | 4 | 1.3 | 1.1–1.5 | | | | | |
| *Ceratoscopelus maderensis* | 1 | 7750 | 3875 | 100 | 2.9 | 2.2–3.7 | 59.9 | 45.8–75.1 | 1.5 | 0.9–2.0 | 5.60 |
| *Diaphus mollis* | 1 | 2 | 2 | 2 | 1.7 | 1.4–2.0 | | | | | |
| *Merluccius bilinearis* | 1 | 73 | 37 | 32 | 12.9 | 11.0–15.4 | 269.2 | 229.5–321.5 | 120.0 | 70.0–210.0 | 3.94 |
| *Stomias boa* | 1 | 2 | 1 | 0 | | | | | | | |
| *Urophycis tenuis* | 1 | 1 | 1 | 1 | 10.0 | 10.0 | | | | | |
| *Loligo* spp. | 1 | 5 | 4 | 1 | 1.8 | | 144.9 | | 67.4 | | 0.07 |
| Total | | 7847 | 3928 | | | | | | | | 9.61 |
| Strandings winter (29 stomachs) | | | | | | | | | | | |
| *Ammodytes* spp. | 1 | 541 | 271 | 32 | 2.2 | 1.9–2.9 | 140.9 | 124.8–172.2 | 15.1 | 10.3–29.0 | 4.09 |
| *Limanda ferruginea* | 1 | 6 | 3 | 0 | | | | | | | |
| *Melanogrammus aeglefinus* | 1 | 8 | 4 | 8 | 11.6 | 10.5–12.5 | 249.7 | 212.2–279.7 | 152.1 | 90.0–210.6 | 1.22 |
| *Merluccius bilinearis* | 2 | 31 | 18 | 15 | 5.2 | 3.9–6.3 | 107.3 | 81.1–131.3 | 7.0 | 2.7–12.5 | 0.13 |
| *Myxine glutinosa* | 6 | 53 | 16 | NA | | | | | | | |
| *Osmerus mordax* | 2 | 20 | 10 | 0 | | | | | | | |
| *Tautogolabrus adspersus* | 1 | 16 | 9 | 16 | 1.0 | 0.9–1.2 | | | | | |
| *Ulvaria subbifurcata* | 1 | 2 | 1 | 2 | 2.5 | 2.5 | | | | | |
| *Bathypolypus bairdii* | 2 | 2 | 2 | 2 | 2.1 | 1.4–2.8 | | | | | |
| *Illex illecebrosus* | 1 | 4 | 2 | 2 | 3.7 | 3.7–3.8 | 191.7 | 189.2–194.1 | 135.1 | 130.3–139.8 | 0.27 |
| *Loligo pealeii* | 4 | 28 | 16 | 12 | 1.6 | 0.4–2.9 | 126.5 | 15.7–246.4 | 89.3 | 0.7–283.6 | 1.07 |
| Total | | 711 | 352 | | | | | | | | 6.78 |

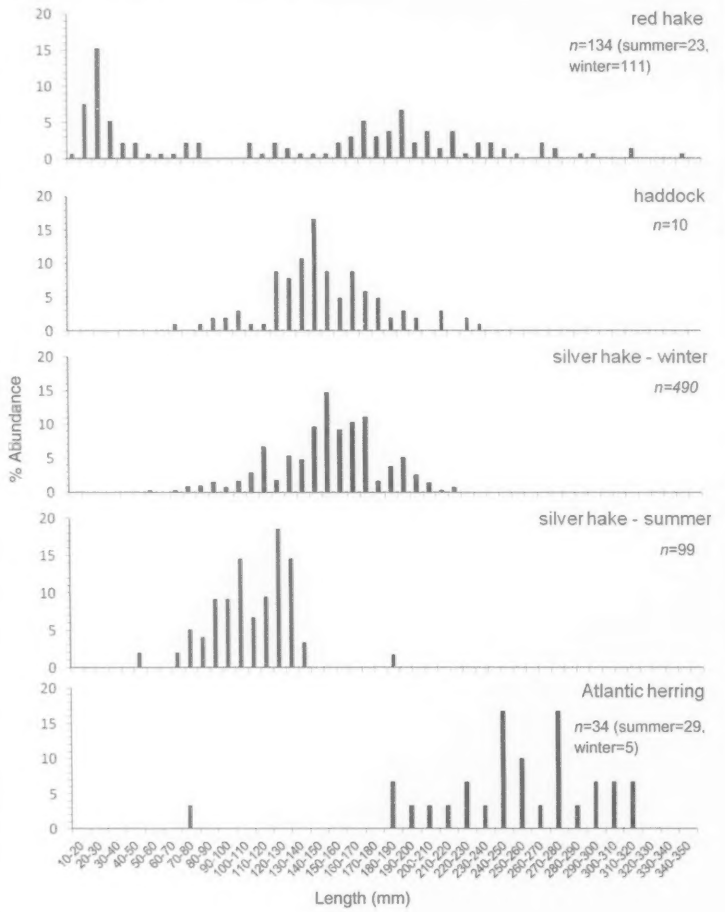


**Figure 2**

Length-frequency distributions for fishes eaten by Atlantic white-sided dolphins (*Lagenorhynchus acutus*) in the Gulf of Maine (1991–2006). (**A**) Red hake (*Urophycis chuss*) all seasons; (**B**) haddock (*Melanogrammus aeglefinus*) all seasons; (**C**) silver hake (*Merluccius bilinearis*) winter; (**D**) silver hake summer; (**E**) Atlantic herring (*Clupea harengus*) all seasons.

frequency diagrams shown in Figure 2 for the most abundant species.

## Results and discussion

Stomachs of immature and adult dolphins of both sexes were examined. The two calves were 119 cm and 139 cm in length and had milk but no solid prey in their stomachs. The smallest juvenile was 161 cm and contained solid prey but no milk in its stomach. Females longer than 200 cm and males longer than 230 cm were considered mature (Sergeant et al., 1980) (Table 1).

Altogether, the 62 *L. acutus* stomachs contained 5561 fishes of at least 26 species, and 304 cephalopods of one octopus and two squid species (Table 2). Most of the stomach contents were trace remains. The most commonly found prey species, in order of abundance, were: 1) Madeira lanternfish (*Ceratoscopelus maderensis*), 3876 individuals, all but one from a single stomach; 2) silver hake (*Merluccius bilinearis*), 945 individuals from 22 stomachs; 3) sand lance (*Ammodytes* spp.), 271 individuals from a single stranded dolphin; 4) spoonarm octopus (*Bathypolypus bairdii*), 212 individuals from 14 stomachs; 5) haddock (*Melanogrammus aeglefinus*), 107 individuals from eight stomachs; 6) Atlantic hagfish (*Myxine glutinosa*), 105 individuals

from 17 stomachs; and 7) red hake (*Urophycis chuss*), 103 individuals from 14 stomachs.

### Gulf of Maine, winter

The 20 stomachs containing prey from *L. acutus* net-caught in winter in the Gulf of Maine provide our most complete data set for analysis (Table 3). These stomachs contained 1155 fishes of 17 species and 274 cephalopods of three species. Five species, three fishes and two cephalopods, silver hake (45.3%), spoonarm octopus (14.3%), haddock (15.3%), red hake (18.7%), and longfin inshore squid (1.1%) accounted for almost 95% of the mass of prey. We were unable to calculate the mass of hagfish; their toothplates (the only hard parts remaining in the stomachs) are not proportional to an individual's size.

In order of frequency of occurrence and numerical abundance 1) the demersal silver hake was found in 90% of the stomachs and accounted for 71% of the fishes eaten; 2) the benthic spoonarm octopus were found in 65% of the stomachs and accounted for 77% of cephalopods; 3) the demersal haddock were found in 35% of the stomachs and made up 8.9% of the fishes; 4) the demersal red hake were found in 65% of the stomachs and accounted for 7.7% of the fishes; 5) the benthic scavenger hagfish were found in 50% of the stomachs and accounted for 7.5% of the fishes; and 6) the pelagic longfin squid were found in 60% of the stomachs and accounted for 23% of the cephalopods. The four fish species constituted 96% of the fish prey eaten, and the two cephalopods made up nearly all of the cephalopods consumed. Fishes made up 81% of all prey, and cephalopods, the remainder (19%). Notable were the scarcity or absence in winter of pelagic fish species (e.g., herring, alewives, smelt, mackerel, and sand lance).

### Gulf of Maine, summer

Only three stomachs were available from animals net-caught in the Gulf of Maine in summer. These three contained 150 fishes of six species and six squids of two species (Table 4). The most abundant prey were silver hake, Atlantic herring, and red hake, which numerically accounted for 63%, 19%, and 8% of total prey, respectively. These three species made up 29%, 64%, and less than 1% of total mass, respectively. Silver hake was numerically the most abundant species in both winter and summer stomachs. The pelagic Atlantic herring, which was almost absent in winter, was the most important species present in summer. The benthic spoonarm octopus, which was very abundant in the winter stomachs, was absent in summer.

### Continental Slope south of Georges Bank

A single *L.acutus* was obtained south of Georges Bank near Munsen Canyon, in 500 m of water. This dolphin was a 252-cm lactating female. Its stomach contained over 3900 fishes of seven species (Table 4). Except for 37 silver hake and one white hake, this individual had been feeding on open-ocean pelagic fishes, of which 99% (3875) were Madeira lanternfish. Two other lanternfishes (the glacier lanternfish [*Benthosema glaciale*] and the soft lanternfish [*Diaphus mollis*]), the white barracudina (*Arctozenus risso*), and the boa dragonfish (*Stomias boa*) were also present. This stomach contained 9.6 kg of prey, the largest amount of any *L. acutus* in this study; Madeira lanternfish made up 58% of the prey mass, and silver hake 41%.

### Strandings

Stomachs of the stranded dolphins contained little identifiable material (Table 1). No nontrace fish or cephalopods were present in any of these stomachs. About one-quarter of the otoliths (27%) were too digested to be identified or measured and therefore could not be used for further analysis. Of the identifiable otoliths, 87% were those of sand lance, all from one stomach (Table 2). The presence of these fish in that stomach was the only evidence indicating recent feeding.

Twenty-nine of the 34 strandings occurred in the winter. Stomachs of these dolphins contained eight fish species and three cephalopod species. Sand lance constituted 79% of all prey (both fishes and cephalopods) and made up 60% of the total mass. Silver hake and longfin squid made up 18% and 16% of the total mass, respectively. Five fish species present in the stomachs of stranded animals—cunner (*Tautogolabrus adspersus*), radiated shanny (*Ulvaria subbifurcata*), rainbow smelt, sand lance, and yellowtail flounder (*Limanda ferruginea*)—were not found in the stomachs of our net-caught dolphins. Of the five stomachs from nonwinter strandings, four were empty and the fifth (in September) contained only one identifiable fish, a silver hake.

### Prey species

In the Gulf of Maine, silver hake was by far the most important prey species in the stomachs that we examined. A schooling, demersal fish, silver hake is found only along the Atlantic coast of North America, from Florida to Newfoundland, at a wide range of depths, from shallow waters to 900 m (Collette and Klein-MacPhee 2002; Iwamoto 2002). Previously, reports indicated the presence of silver hake in *L. acutus* diets but not as an important prey. Among the few otoliths found in stranded *L. acutus* stomachs, silver hake was the second most abundant prey species (Table 4).

Silver hake is also an important prey for other marine mammals in the Gulf of Maine. It was a primary prey for harbor porpoise (*Phocoena phocoena*) during summer in the Bay of Fundy (Recchia and Read, 1989) and in autumn near Jeffreys Ledge in the western Gulf of Maine (Gannon et al., 1998). It was also the predominant prey for net-caught young of the year harbor seals (*Phoca vitulina*) taken in shallow waters in the western Gulf of Maine (Williams, 1999).

Atlantic herring was present in stomachs of both winter and summer net-caught dolphins. It was of minor importance in the winter (only five individuals identified in 20 stomachs analyzed), but was of primary importance (the largest biomass of all prey) in the summer; 29 individuals were present in the three stomachs analyzed. *Lagenorhynchus acutus* feeds on Atlantic herring in the summer months when the lipid content of herring is highest (Yasui and Gaskin, 1986). Even though only three stomachs from the summer were analyzed, our findings are similar to those reported in the literature.

*Lagenorhynchus acutus* is rarely observed in the deeper waters of the continental slope south and east of New England (Selzer and Payne, 1988; Waring et al., 2008). The stomach contents of our single *L. acutus* taken incidentally in water 500 m deep, south of Georges Bank, were unlike those from the Gulf of Maine. The primary prey of this dolphin was the Madeira lanternfish, a myctophid living between 330 and 600 m during the day in slope water and on the continental slope (Backus et al., 1968). This indicates that *L. acutus* is not limited to feeding on shallow-water and epipelagic species.

We have found that other species of cetaceans, when on the continental slope off the east coast of the United States and southeastern Canada, feed primarily on Madeira lanternfish. We found large numbers, often more than 1000 individuals, in common dolphin (*Delphinus delphis*), Atlantic spotted dolphin (*Stenella frontalis*), and pantropical spotted dolphin (*Stenella attenuata*), which had maximum numbers of 3646, 568, and 870 Madeira lanternfish, respectively. A single harbor porpoise (*Phocoena phocoena*) taken incidentally off Cape Hatteras in winter had nearly 500 Madeira lanternfish in its stomach (Read et al., 1996).

In the eastern North Atlantic Ocean, *L. acutus* feeds for the most part on oceanic fishes, primarily silvery pout (*Gadiculus argenteus*), lanternfishes, and pearlsides (*Maurolicus muelleri*) (Couperus, 1997). Couperus found that, in certain years, southwest of Ireland, *L. acutus* follow the spawning migration of mackerel (*Scomber scombrus*) inshore in the late winter and spring and are caught by net in the mackerel fishery. A comparison of trace and nontrace prey in their stomachs indicated that although mackerel represented 88% of the fresh prey, the dolphins' prior meals had comprised 88% mid-water fishes. *Lagenorhynchus acutus* feeds on herring when in shallow water off Norway (Jonsgård and Nordlii, 1952).

In addition to the above mentioned species, stomachs of *L. acutus* stranded on the coast of northern Europe have contained otoliths of blue whiting (*Micromesistius poutassou*), tacauds (*Trisopterus* spp.), horse mackerel (*Trachurus trachurus*), pilchard (*Sardina pilchardus*), sand lances, pollock (*Pollachius virens*), whiting (*Merlangius merlangus*), haddock, gobies (Gobiidae), dragonet (Callionymidae), and argentine (*Argentina sphyraena*), (Desportes, 1985; Rogan et al., 1997; Santos et al., 1995[1], 1996[2]). Santos et al. (1995) also identified four species of cephalopods in the stomachs of *L. acutus* stranded in Scotland.

Despite previous observations of *L. acutus* feeding on sand lance (Weinrich et al., 2001), in this study we found sand lance in only one stomach, that of a stranded dolphin. Although approximately 50% of the stranded animals in our study had virtually empty stomachs, this one stomach contained 549 sand lance otoliths. The relatively empty stomachs indicate a lack of feeding before stranding. Little information exists on the digestion and egestion rates for cephalopod and teleost prey in cetaceans; therefore, it is impossible to accurately estimate how long a period elapsed between the last feeding and the stranding event (Mintzer et al., 2008). It is also possible that *L. acutus* regurgitates food because of the stress of a stranding event, leaving its stomach virtually empty. The trace sand lance otoliths found in the one stomach could have been trapped in the stomach rugae, giving a false impression of a recent meal. Regardless of when the sand lances were eaten, their presence in the stomach corroborates historic observations of *L. acutus* feeding on sand lance (Katona et al., 1978; Weinrich et al., 2001).

*Lagenorhyncus acutus* appears to exhibit size-selective predation; the average fish prey length is approximately 200 mm and cephalopod prey mantle length is about 50 mm during the winter (Fig. 2). The total lengths of the cephalopods (tentacle length plus the mantle length) are little more than half the length of the fishes. One large 464-mm silver hake, whose size was determined from a 22.4-mm otolith and two 100 mm dentaries, was found in the stomach of a winter net-caught dolphin. We assume that the dolphin ate only the head of this fish, which would itself have been approximately 200 mm. It is possible that the head had been cut off and discarded by fishermen.

Some very small red hake (less than 90 mm) were found in the stomachs of two *L. acutus* (Fig. 2A). These were smaller than the smallest silver hake, haddock, and herring eaten, and are therefore evidence of secondary consumption. There were no large red hake or haddock in either of the two stomachs. Large silver hake, the only predatory fish in each of the two *L. acutus* stomachs, was therefore the likely primary consumer of the small red hake.

Two cephalopods, spoonarm octopus and longfin inshore squid, were both important prey in our *L. acutus*. In winter stomachs, the benthic octopus was the second most abundant prey species, and the pelagic squid was the sixth most abundant (Table 3). Historical publications document northern shortfin squid in *L.*

[1] Santos, M. B., G. J. Pierce, G. Wijnsma, H. M. Ross, and R. J. Reid. 1995. Diets of small cetaceans stranded in Scotland 1993–1995. ICES Council Meeting (C.M.). 1995/N:6, 9 p.

[2] Santos, M. B., G. J. Pierce, A. Lopez, A. Barreiro, and A. Guerra. 1996. Diets of small cetaceans stranded in NW Spain 1994–95. ICES Council Meeting (C.M.). 1996/N:11, 6 p.

*acutus* diets, but give no record of spoonarm octopus or longfin inshore squid. Spoonarm octopus was rare (one individual in 95 stomachs) in harbor porpoise from the Gulf of Maine (Gannon et al., 1998).

Both Atlantic hagfish and spoonarm octopus are bottom-dwelling species associated with sandy to muddy substrates on the continental shelf and upper slope. Spoonarm octopus is restricted to the western North Atlantic, and is found from Greenland to Florida (Muus, 2002). Atlantic hagfish is found on both sides of the North Atlantic and is bipolar, inhabiting comparable latitudes in the southern hemisphere (Collette and Klein-MacPhee, 2002). *Lagenorhyncus acutus* probably does not dive to the bottom to forage. It probably consumes benthic octopus and hagfish in several ways, namely 1) it may feed on catch unwanted and discarded by fishermen and 2) it may feed on animals that have been forced off the bottom by otter trawls or that have come off the bottom on their own, as with hagfish, to feed on fishes disturbed by otter trawls. Most of our net-caught dolphins (79%) were taken in bottom otter trawls. Spoonarm octopus and Atlantic hagfish were only found in dolphins caught in bottom otter trawls, not from those in sink or drift gillnets. *Lagenorhyncus acutus* feed in the vicinity of nets (Leopold and Couperus, 1995), as do bottlenose dolphin (*Tursiops truncatus*) (Corkeron et al., 1990) and other cetaceans (Fertl and Leatherwood, 1997). This would explain both the capture of *L. acutus* by otter trawls and the occurrence of benthic animals in their diet.

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# Reflex impairment as a measure of vitality and survival potential of Atlantic cod (*Gadus morhua*)

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**Abstract**—Assuring the vitality and survival potential of live-caught Atlantic cod (*Gadus morhua*) is important for improving the sorting of fish before net penning operations designed to hold fish for growth and later market. When Atlantic cod are captured by Danish seine, the most commonly used fishing gear for live-caught fish, they undergo stressors such as forced swimming, net abrasion, and air exposure. Laboratory experiments (at an air temperature of 9°C and water temperature of 8°C) were conducted with the aim of constructing a RAMP (reflex action mortality predictor) curve for prediction of vitality and survival potential in Atlantic cod captured in Danish seines, by varying the levels of these stressors. Atlantic cod exposed to increased duration in air (5–20 min) showed increased reflex impairment and mortality, with 75% mortality at 10 minutes of air exposure. Forced swimming in combination with net abrasion and air exposure did not increase reflex impairment or mortality above that associated with air exposure alone. The Atlantic cod RAMP curves indicated that fish with reflex impairment less than 50% would not show mortality and would likely recover from capture stress.

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In capture-based aquaculture (CBA; Ottolenghi et al., 2004), fish are caught live and held in net pens to supply high-quality fresh fish throughout the year and thereby increase the value of the catch, given a fixed boat quota. Animal welfare issues in CBA arise when handling stress and adaptation costs to new environments are added to the capture stress. Also the duration of stress will increase dramatically compared to that of traditional commercial fishing where the human impacts on fish end with slaughtering immediately after capture. Improving fish welfare in CBA should be achieved by keeping and storing only vital specimens after a sorting process. Operational indicators of vitality and survival potential are therefore needed.

Current sorting criteria include removing specimens showing any visual sign of damage such as injuries from net abrasion and mechanical handling. Also specimens having clear signs of barotrauma such as gas filled eyes (exophthalmia) or overinflated abdomens due to punctured gas-bladders are poor candidates for live storage and should be removed. This practice combined with the use of specialized transport tanks and net pens, and the implementation of careful handling routines and monitoring of water quality have greatly decreased instantaneous and postsorting mortality. Still some delayed mortality occurs in seemingly unharmed specimens because the internal status of the animal is not readily evaluated by visual inspection. Thus there is the potential for improving the sorting process before the transfer of fish to tanks or net pens by developing operational indicators of vitality and survival potential.

To date there are several physiological and behavioral indicators of fish condition available; however, they share the unfavorable attributes of being expensive and labor intensive and are therefore not suited for routine use in commercial fisheries. More importantly, although applicable for determining sublethal stress levels, they show a lack of concordance with mortality outcomes (Davis et al., 2001; Davis and Schreck, 2005). Recently, reflex impairment has been tested as an indicator of vitality and survival potential (Davis and Ottmar, 2006; Davis, 2007). Correlations between stressor intensity, reflex impairment, and increased mortality were found for several species of round fish and flat fish exposed to simulated capture stressors.

The main objective of this study was to test whether reflex impairment could be used as a rapid real-time operational indicator to predict vitality and survival outcomes in fishing operations designed to hold Atlantic cod (*Gadus morhua*, hereafter referred to as "cod") in net pens

**Table 1**

Reflex actions that were consistently present in 20 control (unstressed) Atlantic cod (*Gadus morhua*) and used to measure reflex impairment in fish exposed to stressors (forced swimming, net abrasion, and air exposure) associated with capture by Danish seines.

| Reflex | Description |
|---|---|
| 1. Body flexion 1 | Fish flexed body when placed on a flat surface (sorting table). |
| 2. Body flexion 2 | Fish flexed when restrained. |
| 3. Head complex | Restrained fish presented alternating mouth gaping and operculum flaring, as if gasping for air. |
| 4. Operculum | Restrained fish either flared operculum rigidly open or strongly clamped operculum shut when operculum was lifted with a probe. |
| 5. Gag | Restrained fish gaged when back of throat was stimulated with a probe. |
| 6. Vestibular-ocular response (VOR) | Restrained fish tracked observer when it was rotated around long axis. |
| 7. Tail flexion | Fish held under belly flexed when tail flanks were stroked by hand along long axis. |

for additional growth and later marketing (CBA purposes). The Danish seine is the most commonly used fishing gear for CBA, and the stressors tested in this study, forced swimming, net abrasion, and air exposure, are typical in and inherent to this type of fishing gear. A series of experiments were conducted to construct a RAMP (Reflex Action Mortality Predictor) curve for cod exposed to these stressors and we broadly followed the three step procedures in Davis (in press) to validate reflex impairment as an indicator of vitality.

First we established appropriate procedures for testing fish reflexes by determining consistent reflexes for testing; we established the effect of repeated reflex testing in control fish to test whether cod were distressed by the testing procedure; we determined the recovery time after exposure to a stressor; and we determined the effect of disturbance from repeated netting of fish from a holding tank.

Second we established experimental methods for exposing fish to key stressors and conducted stress experiments to determine the effect of air exposure and the combined effect of forced swimming, net abrasion, and air exposure on reflexes and survival.

Third after observing immediate and delayed mortality, we correlated reflex impairment and mortality by producing RAMP curves for the effect of air exposure and the combined effect of forced swimming, net abrasion, and air exposure.

## Materials and methods

### Experimental fish

Cod were hatched and later reared in net pens at Austevoll Research Station (60°05′17″N 5°15′50″E), Austevoll, Norway. Five months prior to the experiments, fish were moved (after six months in net pens) to indoor ~1600-L green fiberglass tanks with rounded corners (145 cm × 145 cm × 100 cm depth, water depth 80 cm), supplied with aerated sea water (8.0 ±1.0°C), and fed standard commercial diets (Skretting Ltd, Stavanger, Norway) to satiation by using 24-hr disc feeders (1% of total biomass per day) under a continuous daylight regime. A total of 169 fish (31–49 cm total length [TL]) were tested for reflex impairment and mortality experiments. In order to maintain independence of observation no fish was used more than once.

### Step 1: Consistent reflexes and appropriate procedures for reflex testing

A number of reflex actions in cod for potential inclusion in this study were identified based on previous work (e.g., Davis, 2007) and preliminary trial and error screening on fishing vessels and in the laboratory. In order to identify the reflexes for testing and to standardize methods and stimuli to elicit these reflex actions, twenty cod were initially tested. Seven reflexes were found to be consistently present in control fish (Table 1). For subsequent reflex impairment tests, the reflexes were scored as 1 (present) if a vigorous response was evident, or 0 if the response was not present or of weak or questionable strength. Reflex scoring insured consistent, high quality response data (Davis, in press).

To test reflex actions, fish were initially placed on a flat surface, corresponding to a sorting table on a fishing vessel and observed for spontaneous body flexing. Then fish were placed and held in a restraining device (Fig. 1) consisting of two plastic pieces (60 cm length × 10 cm width), each lined with a sponge to contact and hold fish, and connected at one end to form a hinge, while the other end was secured with a Velcro strap. Fish were then observed for body flexion in response to restraint. The restrained fish were then tested for reflexive head movements (a spontaneous alternating head and operculum movement), operculum movement (either tight closure or flaring after having been lifted with a probe), gag in response to throat stimulation, and vestibular-ocular response (eye rotation while the fish was rotated around its long axis). Finally fish were released from restraint and tested for body flexion in

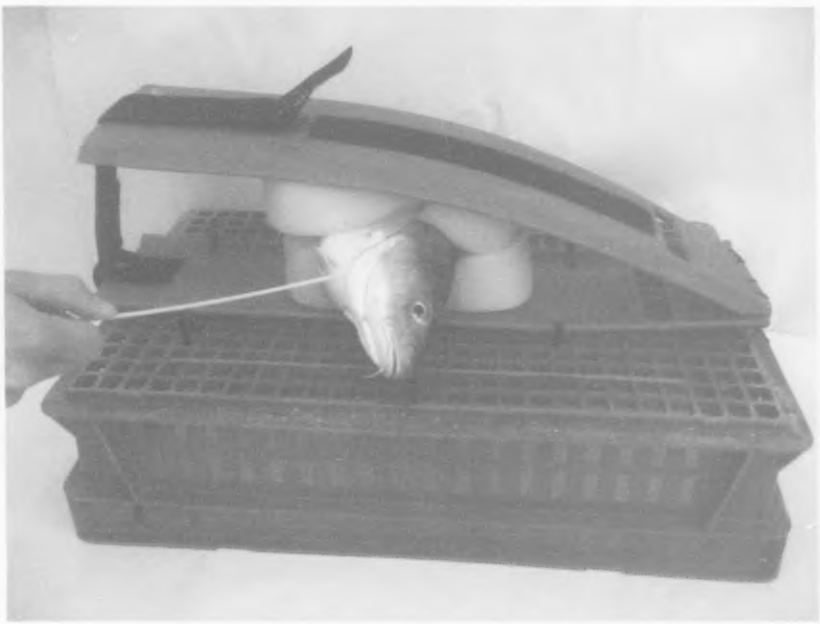

**Figure 1**

Fish restraining device used to test reflex actions in Atlantic cod (*Gadus morhua*). In this photograph, the fish is being tested for operculum reflex action.

response to hand stroking of the tail flanks along the long axis while being held under the abdomen. The entire sequence for reflex testing in a replicate fish was conducted within 60 seconds.

Repeated reflex testing was conducted with a group of ten fish to determine if cod were stressed by the reflex testing procedure itself. These fish were netted, restrained, and their reflexes were tested at 0, 60, and 180 minutes after the initial netting and restraint. Between each sampling time, fish were placed back into a tank with sea water and recaptured for the next reflex test.

The effect of recovery time (0–15 min) on reflexes after exposure of fish to stressors was tested to determine an appropriate time to sample cod reflexes. Replicate fish ($n$=35) were exposed to standard stressors (see detailed description of stressor administrations below in step 2) of 5 minutes of swimming, 1 minute of net abrasion, and 5 minutes in air (all at 9.0°C). Then fish were returned to the stock tank and reflex testing was begun at 0, 5, 7, 10, or 15 minutes after exposure to stressors and completed within 8 minutes from starting time of testing for each exposure group ($n$=7 per group).

It was possible that disturbance from repeated netting of fish from a stock tank before netting replicate fish for stressor treatment could have affected the level of reflex impairment resulting from exposure to stressors, because fish could have been repeatedly stressed by being chased before capture. To test this possibility, fish were exposed to 5 minutes of swimming, 1 minute of net abrasion, and 10 minutes of air exposure (all at 9.0°C) after being either captured from a tank ($n$=8) in which four fish had been previously captured or from a tank ($n$=8) in which 16 fish had been previously captured. Then fish were returned to the tanks and reflex testing began 5 minutes after exposure to stressors and was completed within 9 minutes from the start of testing for each exposure group. Fish were then held to determine mortality. Immediate and delayed mortality resulting from stressors was observed by holding fish in tanks for 60 days and removing and counting dead fish daily (mortality observed as common death signs, e.g., motionlessness, static flaring of operculum, nonresponsiveness to touch stimuli, *rigor mortis*). The rearing regime for the stressed fish was similar to stock tank fish.

### Step 2: Stressors, exposure experiments, and stress induction

Stressors were selected to mimic the stressors cod experience during capture during Danish seine operations and to create a gradient of stress from low to moderate to lethal. Exposure to air was chosen for its known importance as a stressor for fish sorted on deck. Forced swimming is a relevant capture stressor. Net abrasion

is important during haulback operations and transfer of fish from the seine to the boat in CBA. Moderate intensity of abrasion was administered in the experiment because these injuries are likely to occur during sorting and may increase the risk for infection, disease, and delayed mortality. Fish exposed to these stressors (air exposure, forced swimming, moderate net abrasion) may not have obvious external injury and the effects of such stressors may be difficult to assess by visual inspection during sorting. Moreover fish experiencing different levels of such stressors may differ in internal status and survival potential.

Exposure to air was caused by placing fish in a tank without water to simulate conditions that would occur during sorting on deck (on the sorting table) after capture, and the intensity of the stressor was controlled by altering exposure duration. The swimming stressor was created by forcing fish to swim against a current created by lowering the water level in a tank (145 cm × 145 cm × 8 cm water depth) in which water was introduced along the tank wall to cause a circular current of approximately 20 cm/s. This stressor intensity was varied by controlling the amount of time that the fish was forced to swim against the current. Net abrasion was caused by capturing the fish in a net and sloshing them up and down at the water surface in a tank for a fixed time of one minute. After administration of stressors the fish were placed into a stock tank, and one individual at a time was taken out for reflex testing.

The effect of exposure to air alone was tested in cod ($n$=32) exposed to either 5, 7, 10, or 20 minutes of air (all at 9.0°C). After air exposure, fish were placed into a stock tank and then individuals were taken out for reflex testing. Testing began 5 minutes after the exposure to air ended and was completed within 9 minutes from the start of testing for each exposure group ($n$=8 per group). Fish were then held to determine mortality.

The effect of exposure to seven combinations of swimming, net abrasion, and air was determined to simulate stressors associated with capture by Danish seine. Replicate fish ($n$=56) were exposed to swimming for 5, 10, or 15 minutes, net abrasion for 1 minute, and air for 5, 10, or 15 minutes (all at 9.0°C). Then fish were returned to tanks and reflex testing began 5 minutes after exposure to stressors and was completed within 9 minutes from the start of testing for each exposure group ($n$=8 per group). Fish were then held to observe mortality.

### Step 3: RAMP curves and statistics

The proportion of reflex impairment in each fish was calculated as follows: 1 – (the sum of individual reflex scores/the total possible score of 7), i.e,. the proportion of reflex impairment was 2/7 if five out of the seven reflex actions (see Table 1) were present. Mortality for individual fish was scored as 1 (present) or 0 (absent) and the mortality date was noted. Statistical significance of correlation between reflex impairment and mortality was tested by using Spearman rank correlation. Sigmoid curves (RAMP curves) showing relationships between reflex impairment and mortality were fitted by using SigmaPlot vers. 10.0 (Systat Software, Inc., Chicago, IL). When control fish reflexes were sampled repeatedly, the effects on reflex impairment were tested with Friedman analysis of variance (ANOVA), whereas the effects of sampling time, air exposure time, forced swimming, net abrasion, and air exposure were tested with Kruskal-Wallis ANOVA. The effect of disturbance on reflexes was tested with a two sample $t$-test. Statistical significance was accepted at $P \leq 0.05$.

## Results

### Step 1: Appropriate procedures for reflex testing

Reflexes in control (unstressed) cod were not impaired by repeated testing at 0, 60, and 180 minutes after initial capture from a stock tank (ANOVA, $F_{2,9}$=0.64, $P$=0.728), indicating that cod were not stressed by the reflex-testing procedure itself. Mean reflex impairment (proportion) for each testing period ranged from 0.03 to 0.06 and no mortality occurred.

Reflex impairment occurred immediately after exposure to 5 minutes of swimming, 1 minute of net abrasion, and 5 minutes of air exposure and was not significantly changed (mean ±1 standard error) by the time of sampling when sampled at 0 (0.71 ±0.08), 5 (0.65 ±0.03), 7 (0.61 ±0.05), 10 (0.50 ±0.07), or 15 minutes (0.51 ±0.07) after exposure to stressors (ANOVA, $F_{34}$=7.92, $P$=0.095). These results indicate that reflex actions are real-time indicators of stress, and recovery does not happen within the first 15 minutes after exposure to stressors.

Repeatedly disturbing fish (16 times) in a stock tank by chasing and netting fish out of the tank before netting fish for stressor treatment did not result in increased mean (±1 standard error) reflex impairment (0.86 ±0.08) or mortality (0.75 ±0.16) after the fish were subjected to 5 minutes of swimming, 1 minute of net abrasion, and 10 minutes of air exposure when compared to repeatedly disturbing fish (4 times) (reflex impairment=0.82 ±0.08; mortality=0.50 ±0.19), after the fish were exposed to the same stressors (reflex impairment $t$-test, $t_{14}$=0.32, $P$=0.751; mortality $t$-test, $t_{14}$=1.00, $P$=0.334).

### Step 2: Exposure experiments

Cod exposed to increased duration in air (5, 7, 10, and 20 minutes) showed increased reflex impairment (ANOVA, $F_{31}$=17.11, $P < 0.001$; Fig. 2). Mortality also increased with increased air exposure (ANOVA, $F_{31}$=17.87, $P$<0.001; Fig. 2). Air exposure was an important stressor and mortality increased rapidly as the duration of time that fish were exposed to air increased, and 75% mortality was observed at 10 minutes of air exposure. At 20 minutes of air exposure 100% mortality was observed. No immediate mortality was observed from any of the air expo-

sure experiments, whereas 94% mortality (delayed mortality) occurred within 1 day (16 fish) and one fish died after 6 days.

Forcing cod to swim for 5, 10, or 15 minutes at 20 cm/s in combination with net abrasion and air exposure did not increase reflex impairment or mortality above that associated with air exposure alone. Reflex impairment increased with increased exposure to a combination of swimming, net abrasion, and air, and this effect appeared to be associated primarily with increased duration in air (ANOVA, $F_{55}$=28.82, $P$<0.001; Fig. 3). Likewise, mortality increased with increased exposure to these stressors, and mortality appeared to be primarily associated with air exposure (ANOVA, $F_{55}$=25.15, $P$<0.001; Fig. 3). Cod injury from net abrasion was not obvious, other than the presence of sloughed scales on the net. No immediate mortality was observed from any of the combined stressors, whereas 94% of observed delayed mortality occurred within 1 day (15 fish) and one fish died after 14 days.

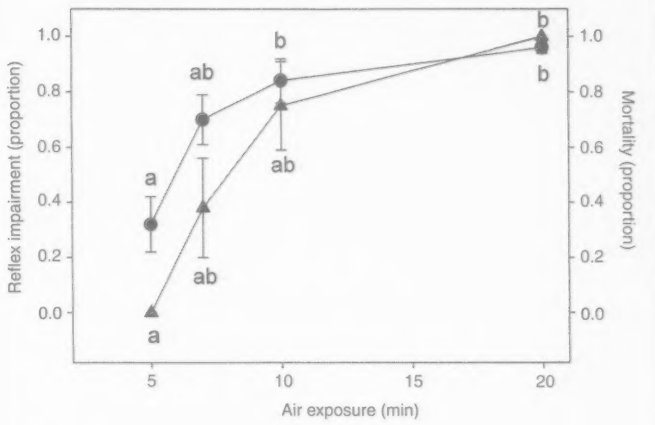


**Figure 2**

Atlantic cod (*Gadus morhua*) mean proportion of reflex impairment (●) and mortality (▲) for treatment groups ($n$=8 fish per treatment group) increased with increased duration (minutes) of exposure to air. There were two groups of mean (±1 standard error) values that were significantly different (indicated by a, b).

### Step 3: RAMP curves

Reflex impairment and mortality were correlated for individual cod exposed to air (Spearman rank correlation=0.85, $P$<0.001, $n$=32) and when exposed to swimming, net abrasion, and air (Spearman rank correlation =0.85, $P$<0.001, $n$=64). The relationship between reflex impairment and mortality was sigmoid (RAMP curve), initially showing increasing reflex impairment with no mortality, followed by a sharp increase in mortality at reflex impairment values > 0.6 (Fig. 4A). The relationship between reflex impairment and mortality calculated for cod in groups defined by experimental treatments that combined swimming, net abrasion, and air exposure (Fig. 4B) showed a similar pattern to that shown by the RAMP curve for individual fish, indicating that reflex impairment could predict mortality in populations of cod, as well as in individual fish.

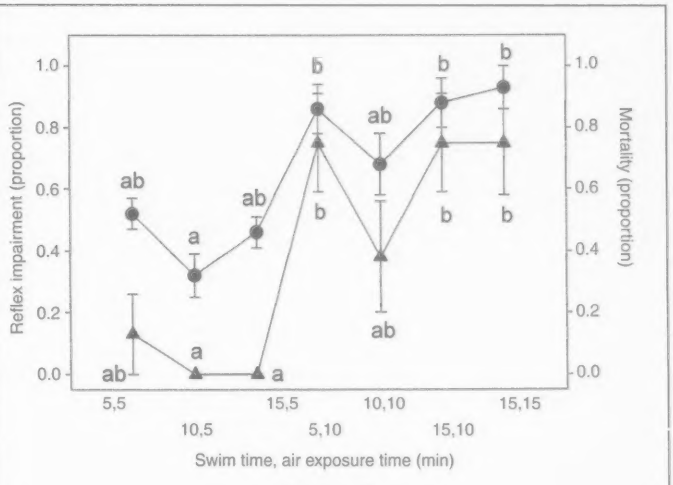


**Figure 3**

Atlantic cod (*Gadus morhua*) mean proportion of reflex impairment (●) and mortality (▲) for seven treatment groups ($n$=8 fish per treatment group) increased with increased stressor intensity. Fish were exposed to combinations of swimming (5, 10, and 15 minutes), net abrasion (1 minute), and air (5, 10, and 15 minutes). There were two groups of mean (±1 standard error) values that were significantly different (indicated by a, b).

## Discussion

Capture-based aquaculture (CBA) is a combination of capture by commercial fisheries and rearing by aquaculture. In this article we address key stressors associated with the capture portion of CBA. Stress and mortality in fish from commercial fisheries often result from several classes of interacting acute stressors. These classes include capture stressors (net entrainment, mesh passage, crushing, wounding, sustained swimming until exhaustion, and pressure changes), fishing conditions (towing time, light

conditions, water and air temperatures, hypoxia, sea conditions, time on deck, and handling procedures), and biological attributes (behavior, size, and species) (Davis, 2002). This study demonstrated that reflex impairment can be used to assess vitality and survival potential of cod exposed to capture related stressors such as forced swimming, net abrasion, and air exposure. Cod showed reflex impairment immediately after exposure to stressors, suggesting that reflex actions are sensitive real time indicators that integrate neurological, hormonal, and behavioral states that can be related to changes in fish vitality and welfare (Davis, in press). Importantly, the testing of reflex actions did not cause reflex impairment, indicating that cod were not stressed by the procedure. Also, disturbances caused by repeated netting of fish from the holding tank did not affect the level of reflex impairment resulting from exposure to stressors.

The RAMP curves for individuals and groups indicated that cod with less than 50% reflex impairment would not die and would likely recover from capture stress to resume normal feeding and growth. Similar correlations between stressor intensity, reflex impairment, and increased mortality were found for several species of roundfish and flatfish exposed to simulated capture stressors (Davis and Ottmar, 2006; Davis, 2007). Future measurements of reflex impairment and mortality in cod caught by Danish seine and held in net pens can be used to validate the laboratory RAMP curves for use in CBA operations. Experiments could be designed to expose cod to gradients of capture-related stressors in the field, to test for reflex impairment, and then to hold fish in the field in order to determine delayed mortality. Reflex impairment and mortality results from field stressor experiments could be compared with mortality rates that were predicted with the laboratory RAMP curve for cod. Possible differences in prediction of mortality rates between laboratory- and field-derived RAMP curves may be evident as a shift in the relationship between reflex impairment and mortality. These difference would be likely caused by the addition of stressor types in Danish seine operations that were not included in the laboratory experiments. In general the types of stressors and not the intensity of stressors control the shape of the RAMP curve (Davis, in press). The RAMP curves could also be applied to estimate survival of escapees and discarded fish (e.g., Davis and Ottmar, 2006; Ingolfsson et al., 2007; Enever et al., 2009) and to improve selection of cod with high survival potential in tagging studies (Fowler and Stobo, 1999; Brattey and Cadigan, 2004). Also our result for time of delayed mortality is of interest in relation to tagging studies; because 94% of delayed mortality occurred within 24 hours, holding fish for one day before release should reduce tagging mortality to negligible levels.

Exposure to air should be avoided when possible for cod, but if inevitable, it should be kept below safe levels. Air exposure was the most important stressor associated with mortality in cod with 40% mortality observed at 7 minutes, 75% at 10 minutes, and 100% at 20 minutes (Fig. 2). However, during the reflex testing procedures, air exposure of one minute or less did not induce reflex impairment or mortality and thus represents a conservative, safe level of ex-

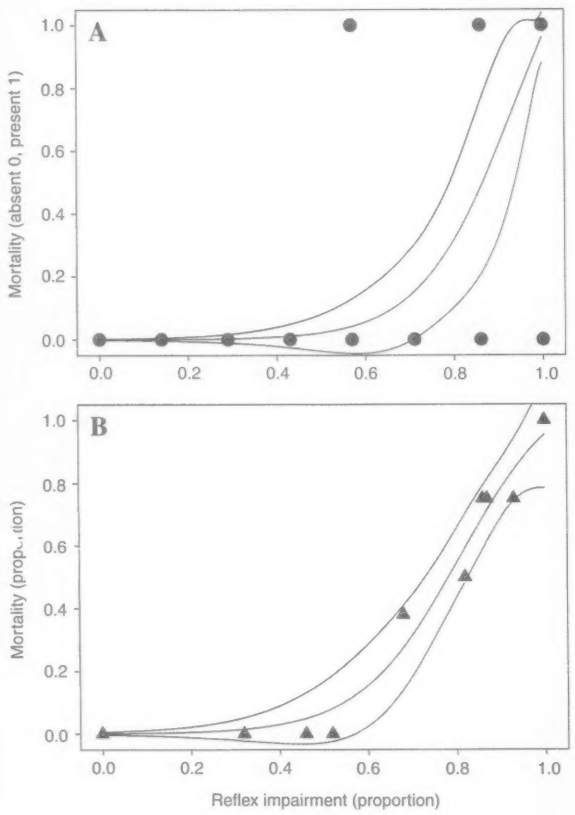


**Figure 4**

Reflex impairment (proportion) could predict (**A**) mortality (absent 0, present 1) in individual Atlantic cod (*Gadus morhua*), or (**B**) mean mortality (proportion) in populations of cod exposed to combinations of forced swimming, net abrasion, and air (see *Methods* section). For individual (●) cod in (A), the sigmoid curve ($y=1.471/(1+e^{-(x-0.935/0.104)})$ ±95 % confidence intervals was significant ($r^2=0.84$, $P<0.001$, $n=64$). Note that many points overlap. For populations (▲) of cod (B), defined by calculating mean proportions for reflex impairment and mortality in ten combination stressor treatment groups (including control and 100% mortality groups), the sigmoid curve ($y=1.130/(1+e^{-(x-0.809/0.113)})$ ±95 % confidence intervals were significant ($r^2=0.97$, $P<0.001$, $n=10$). Note that two points overlap.

posure. Sensitivity to air exposure has been shown to vary among species, with mortality occurring at 7 to 45 minutes of exposure (Davis, 2002). Air exposure is often inevitable during sorting in live-capture operations, and duration in air can be reduced to safe levels, e.g., by the introduction of water-filled sorting tables.

Increased temperature is another stressor that has been associated with mortality in captured fish and may become important if cod are captured or sorted at temperatures above 12°C (Davis, 2002; Sartoris et al., 2003; Brattey and Cadigan, 2004; Suuronen et al., 2005). Increased temperature may also raise sensitivity to air exposure (Davis and Parker, 2004; Davis and Schreck, 2005). Temperatures in deep (200 m) and shallow (surface) water peak in July and August (at ~3 and ~8°C, respectively) in coastal areas suitable for CBA in northern Norway (Loeng, 1991). During spring months when capture for CBA purposes occurs, mixing in these areas is high, and temperatures remain around ~3°C throughout the water column and air temperature only rarely raises above 12°C in May (data from The Norwegian Meteorological Institute, Blindern, Oslo). Temperature should thus not be a critical factor at the temporal and spatial scales of current CBA practices in Norway, and accordingly we did not include temperature as a variable in our study. However attempts to develop CBA outside this temporal and spatial window (e.g., North Sea during summer months), warrant a high vigilance of temperature effects.

A likely effect of adding a stressor is that it may cause an increase in the overall stress response. However, forcing cod to swim for 5, 10, or 15 minutes at 20 cm/s in combination with net abrasion and air exposure did not increase reflex impairment or mortality above that associated with exposure to air alone. This result even indicated that swimming for 10 minutes may have enhanced resistance to air exposure, having a palliative effect on the induction of further stress. In a study of free swimming cod, oxygen consumption did not increase until fish were swimming at 30 cm/s or higher, indicating that at less than 30 cm/s cod would not be stressed (Claireaux et al., 1995). Although not directly relevant to this study, sustained slow swimming in rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*) has reduced the duration of recovery from exhaustive exercise (Farrell et al., 2001; Lee-Jenkins et al., 2007). Little is known about the palliative effects of low-intensity exercise in fish, either before or after exposure to stressors. Studies of the swimming performance of cod have been focused on measurements of endurance and burst swimming (40–130 cm/s) and have included an evaluation of changes in the scope for metabolic activity (Reidy et al., 2000). Further study of the interactive effects of low-intensity exercise, the perception of stressors by fish, and their management of stress may contribute to methods for the significant reduction of stress in captured, released, transported, and net-penned fish.

Injury to cod from net abrasion was not obvious, other than the presence of sloughed scales on the net, and did not appear to contribute to delayed mortality or reflex impairment. Fish with obvious damage to the skin, fin erosion (split fins), cataracts or opaque eyes, resulting from severe abrasion from net material will not pass the sorting procedure in CBA. Therefore a moderate intensity of abrasion was administered in this experiment because these injuries are not likely to be detected during sorting and may increase the risk for infection, disease, and delayed mortality. Such effects of moderate net abrasion were, however, not demonstrated in our experiment. Mortality rarely occurred in cod that were injured by net abrasion when escaping from a demersal trawl (Soldal et al., 1993). Injury to cod from fishing gear can occur, however; Baltic cod have been observed with a high incidence (48%) of skin infection probably associated with escape from fishing gear, but associated mortality was not determined (Mellergaard and Bagge, 1998). In other studies of net abrasion and consequent mortality, some species (herring, [*Clupea harengus*], and walleye Pollock [*Theragra chalcogramma*] were sensitive to net abrasion and showed delayed mortality associated with skin infection (Suuronen et al., 1996; Davis and Ottmar, 2006), whereas other species (sablefish [*Anoplopoma fimbria*] and Pacific halibut [*Hippoglossus stenolepis*] were more resistant and mortality was not correlated with skin abrasion (Davis and Ottmar, 2006).

Capture, transport, and holding of fish are often associated with induction of stress and reduction of vitality. Reflex impairment could be used to evaluate the role of different stressors at each stage of the live-fish fishery and to identify fish with the highest probability for survival. Successful live-fish capture, transport, and rearing operations should aim to minimize stress, optimize water quality, and minimize the increase of metabolic wastes (Huntingford et al., 2006; Ashley, 2007). Water quality and temperature must be controlled through inputs, tank and pen configurations, and flow rates. Food (e.g., Olsen et al., 2008) and stocking density (Staurnes et al., 1994) may be important factors because they control physiological and behavioral states of fish. The adjustments of wild fish to confinement and unnatural densities and how these short-term adjustments affect future performance and welfare are also important considerations. Reflex testing may be performed also in free-swimming fish (Davis and Ottmar, 2006; Stien et al., 2007), and monitoring reflex impairment in captive fish can be a rapid real-time method for identifying optimal transport and rearing conditions and for tracking recovery in cod after live capture.

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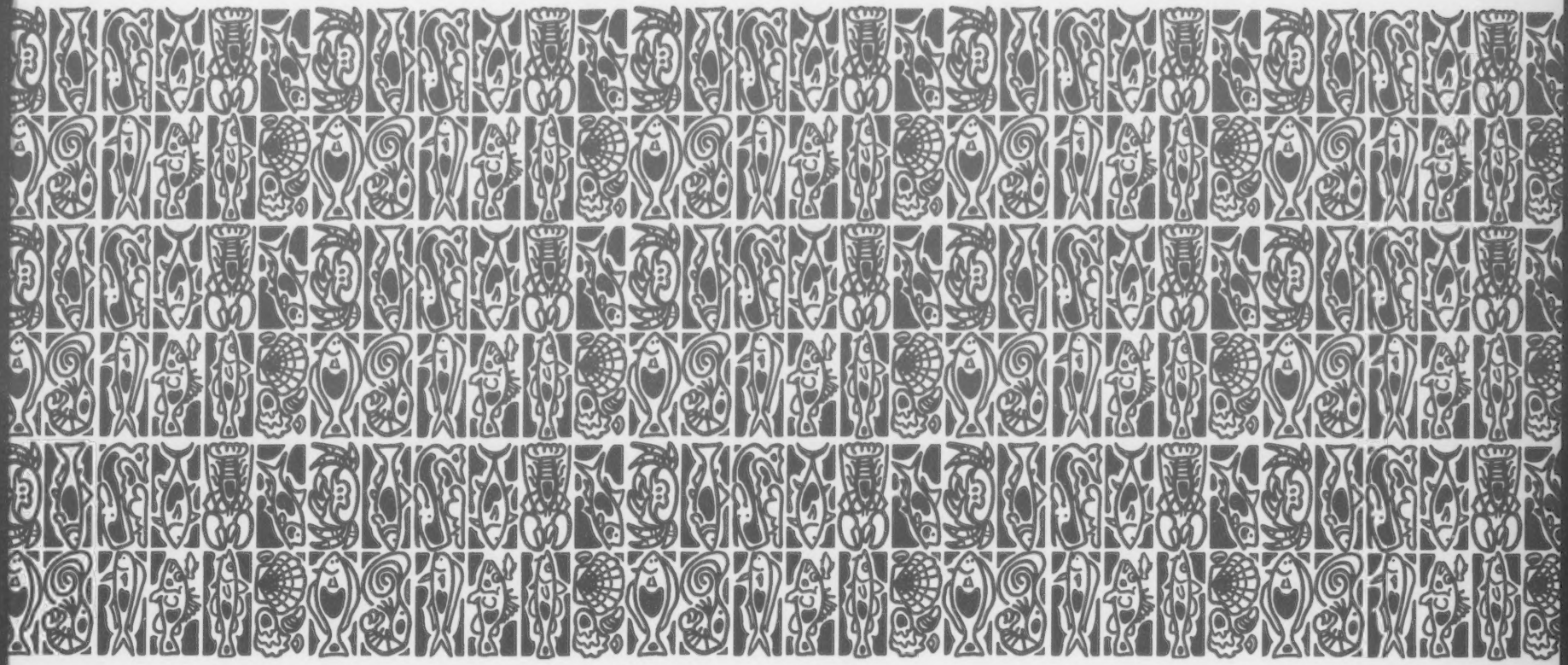
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